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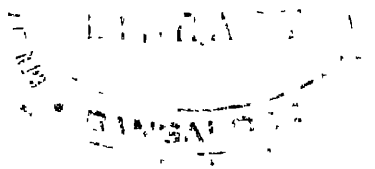

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HYDROGEN ION CONCENTRATION
OF THE
BLOOD IN HEALTH AND DISEASE

MEDICINE MONOGRAPHS

VOLUME VIII

HYDROGEN ION CONCENTRATION OF THE BLOOD IN HEALTH AND DISEASE

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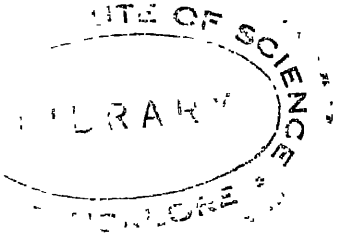

FOREWORD

The development of methods for measuring hydrogen ion concentration has led to many studies of the significance of variations of the hydrogen ion concentration of the blood in health and in disease. It seems desirable at this time to bring together the most important facts in a concise form for the use of clinicians and workers in our clinical laboratories. The importance of this is the greater since the study of hydrogen ion concentration serves as an introduction to the study of other ion activities in biological fluids, a field to which attention is now being directed.

This survey of the subject is not intended as an exhaustive review with critical analysis of all work on the subject. It is planned rather to include only that work, which carried out with the more recently developed technique, is at the same time accurate and of importance in clinical medicine.

CHAPTER I

GENERAL CONSIDERATIONS

The following discussion of the theoretical and practical considerations involved in the measurement of the hydrogen ion concentration and its significance in body fluids is intended only as a convenient outline for those readers whose interests have been clinical rather than physiological. The electrolytic theory is utilized in its simplest form, regardless of the present question of complete and partial dissociation and we use the familiar term hydrogen ion concentration rather than the less familiar though more accurate term, activity. Only at one point in the paper have we used the term "activity"; namely in referring to the Donnan equilibrium. In this instance it seems to us of especial importance to keep in mind the distinction between thermodynamic activity and chemical concentration.

In aqueous solution certain substances, acids, bases and their salts, are changed to greater or less degree, into components which carry an electric charge which are called ions. This process of electrolytic dissociation may be represented by the equation

$$HA \rightleftarrows H^+ + A^- \qquad (1)$$

This phenomenon is of the utmost importance in life processes and especially so in the complex mammalian organism because the processes of digestion, respiration and excretion are all to a great extent concerned with substances which in solution dissociate into electrolytes. Of immediate interest to this discussion are the acidic and basic substances which are present in the body fluids.

Two points are of especial importance: (1) that this reaction is a reversible one and that the undissociated molecules are in continual equilibrium with the ions, and (2) the ions as well as the undissociated molecules for the purposes of this discussion may be assumed, in the concentration met with in blood, to obey the law of mass action.

MEASURE OF ACIDITY IN SOLUTIONS

In measuring the alkalinity or the acidity of any solution two factors must be considered, the *quantity* of the acidic or basic substance and the *intensity* of the degree of acidity or alkalinity. The quantity factor is expressed in terms of the concentration of the substance in chemical terminology as the normality or the number of gram equivalents per liter.

The *intensity* factor, the degree of acidity or alkalinity, often referred to as the *reaction* of the solution, depends upon the H^+ and OH^- ion concentrations and is expressed usually in terms of the normality of hydrogen *ions*.

A most important property of aqueous solutions is that the concentrations of H^+ and OH^- ions are related to each other thus:

$$[H^+] \times [OH^-] = K_w \qquad (2)$$

where for any given temperature K_w is a constant and when $[H^+]$ increases $[OH^-]$ decreases and vice versa, so that it is possible at any one temperature to express both acidity and alkalinity in terms of either ion. It is usual to use the $[H^+]$ for this purpose and to speak of the hydrogen ion concentration of the solution. Throughout this paper we will use a bracketed chemical symbol to indicate the concentration (or activity) of a substance in solution.

The relation between the intensity and the quantity factor of acidity is analogous to the relation between the intensity and quantity factors in electrical energy. Electromotive force is the intensity factor which determines whether or not current flows and the quantity factor, ampère, is the measure of the total amount of current. This relation may also be compared to differences between temperature and calories, or to the older analogy between the pressure of water, due to the height of the reservoir, and the content of the reservoir.

Another interesting comparison between the intensity factors $[H^+]$ and t° is that the zone of $[H^+]$ compatible with life is as small compared to known $[H^+]$ concentrations as is the zone of temperature compatible with life to known temperatures.

In biological solutions the normality of $[H^+]$ is so small that the fractional values are difficult to visualize. Furthermore, in deter-

mining and plotting $[H^+]$ values it is often convenient to use logarithms. Soerensen (1909) therefore introduced the term pH as a convenient symbol for a measure of $[H^+]$ concentration, defined as follows:

$$pH = \log\left(\frac{1}{[H^+]}\right)$$

where "p" means "exponent" (potenz).

The relation of pH to $[H^+]$ is most clearly shown by examples. An N/10 HCl solution, assuming it to be completely dissociated into H^+ and Cl^- ions, will also be tenth normal with respect to hydrogen ions, or

$$[H^+] = 1/10\ N = 0.1\ N = 1 \times 10^{-1}\ N$$

or

$$\text{its } pH = 1.0$$

In like manner the hydrogen ion concentration of a serum may be expressed thus

$$H^+ = 0.000{,}000{,}032\ N = \frac{0.32}{10^7}\ N = 0.32\ N \times 10^{-7}\ N = \frac{1}{10^{7.5}}\ N = 10^{-7.5} N$$

or

$$\text{its } pH = 7.5$$

In using pH it is necessary to remember that change of 1 pH unit indicates a 10-fold change in H^+ concentration, that increase in pH means decrease in $[H^+]$ and that pH 7.0 at 20° and 6.8 at 38° represent neutrality. Thus a decrease of pH from 7.4 to 6.4 means that the H^+ concentration has become 10 times *greater*, and that at either 20° or 38° the solution instead of being slightly alkaline, has become slightly acid.

RELATION BETWEEN $[H^+]$ AND GRAM EQUIVALENT NORMALITY OF ACID CONCENTRATION

Since $[H^+]$ is the measure of the *intensity* factor, and titration of the *quantity* factor, it follows that for any solution the difference between these two measurements is dependent upon the degree of dissociation. With an almost completely dissociated acid, as tenth or hundredth normal HCl solution, the two measurements expressed in

normalities agree but in the case of many acids as carbonic, phosphoric, acetic, etc., the dissociation is low and the $[H^+]$ normality is therefore only a small fraction of the gram equivalent normality of total acid concentration.

In such solutions therefore the titration values give no indication of the actual $[H^+]$ concentrations of the solutions.

RELATION OF $[H^+]$ TO BUFFER EFFECT

Let us now compare the effect of adding strong acid to solutions of salts of strongly and weakly dissociated acids. If we add 1 cc. of N/10 HCl to (1) a liter portion of 0.15 N sodium chloride (physiological saline) and (2) to a liter portion of 0.15 M sodium phosphate solutions both at neutrality (pH = 7), the resulting pH of the NaCl solution will become about 4, a 1000-fold increase in acidity, while the pH of the phosphate solution will be practically unchanged. The importance in biology of substances, whose solutions show comparatively small changes in $[H^+]$ with large addition of acid or alkali was pointed out by L. J. Henderson (1908) and to them Soerensen (1909) gave the name "buffer" substances.

The hydrogen ion concentration of body fluids in general and of the blood in particular is kept within rather narrow limits by means, in large measure, of such buffer systems. In the blood the systems are in order of importance, hemoglobin acid and its salt, carbonic acid and alkali carbonate, serum proteins and their salts, and monobasic and dibasic phosphate. In this discussion the proteins including hemoglobin will be considered to be dissociated as weak acids (see Loeb, 1922). The $[H^+]$ of a mixture of a weak acid with its salts may be calculated by the equation

$$[H^+] = K \frac{[\text{free acid}]}{[\text{salt}]} \tag{3}$$

Thus for serum

$$[H^+] = K_1 \frac{[H_2CO_3]}{[BHCO_3]} = K_2 \frac{[BH_2PO_4]}{[B_2HPO_4]} \tag{4}$$

where K_1 and K_2 represent the dissociation constants for the individual systems and B represents sodium or potassium.

In logarithmic form this may be written to include also protein as follows:

$$pH = pK_1' + \log \frac{[BHCO_3]}{[H_2CO_3]} = pK_2' + \log \frac{[B_2HPO_4]}{[BH_2PO_4]} = pI + \frac{[BPr]}{\beta[Pr]} \quad (4a)$$

The expression

$$pH = pI + \frac{[BPr]}{\beta[Pr]} \quad (4b)$$

is a rearrangement of Van Slyke's buffer equation

$$[BPr] = \beta[Pr]\ (pH - pI) \quad (4c)$$

where [BPr] = milli-equivalents of base bound by protein.

β = the buffer constant for the particular serum or blood; *i.e.*, the change in milli-equivalents of base bound by 1 unit of [Pr] for 1 unit ΔpH.

[Pr] = the concentration of protein for the serum or blood. For serum protein the unit of [Pr] is 1 gm. For hemoglobin it is 1 millimol.

[pI] = an empirical constant that for a single protein is close to but not necessarily identical with the pH at the isoelectric point.

L. J. Henderson (1908) first showed that the carbonate and phosphate equilibria of this equation are applicable to the blood system. In 1916, Hasselbalch used the equation in the logarithmic form and gave values for pK′ in the following equation:

$$pH = \log \frac{1}{[H^+]} = \log \left(\frac{1}{K \dfrac{[H_2CO_3]}{[BHCO_3]}} \right) = \log \left(K' \frac{[BHCO_3]}{[H_2CO_3]} \right) = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]} \quad (5)$$

Several relations, of especial importance in blood may be deduced from the above equations. First, for a given [H+] the *ratios* of acid and salt components to each other are fixed for every system. Secondly, if the ratio for any one system can be determined and if the K values are known the value of [H+] as well as of all the other ratios can be calculated. This fact is the basis for the calculation of pH or of pCO_2 or $[H_2CO_3]$ when one of these and total $[CO_2]$ are known.

A third fact is evident from (4): that [H+] is not dependent upon the concentration of the buffer substance but upon the ratio of concentration of its two components. This is discussed more fully under "Alkali reserve."

THE ACID BASE BALANCE OF THE BLOOD

The reaction of the blood is maintained within narrow limits by a remarkable adjustment to varying conditions. The daily metabolism involves the ingestion of varying quantities of acid and basic salts, the production in metabolism of tremendous quantities of acids and

bases, espcially H_2CO_3 and NH_3 and the neutralization and excretion of these products. The physiological and chemical mechanisms of these processes have been reviewed thoroughly recently by L. J. Henderson (1921), Van Slyke (1921a, 1921b), Barcroft *et al.* (1922) and Wilson (1923) so that we need only refer briefly to factors which are of importance in abnormal conditions.

Under normal conditions the reaction of the blood is stabilized through three main processes in addition to the effects of its buffer: excretion of the non volatile acids and bases through the kidney, change in the base binding properties of hemoglobin with oxygenation and reduction, and excretion of carbon dioxide through the lungs.

The first process is slow compared with the almost instantaneous adjustments secured by the last two.

The amounts of CO_2 formed in the tissue and transmitted to the circulating fluid is so great that if only the buffer action of the blood's buffer systems were available the resulting reaction would pass beyond the limits of acidity compatible with life. However, oxyhemoglobin is a stronger acid than reduced Hb so that as Hb is reduced, which occurs coincidently with increase of CO_2, some of the base B bound with it as $BHbO_2$ may combine with H_2CO_3. This process is reversed in the lungs, thus:

$$\text{Tissue: } BHbO_2 + H_2CO_3 \rightarrow BHCO_3 + HHb + O_2 \quad (6)$$

$$\text{Lung: } BHCO_3 + HHb + O_2 \rightarrow BHbO_2 + H_2CO_3 \quad (7)$$

By such a mechanism the ratio of $\frac{H_2CO_3}{BHCO_3}$ is kept approximately constant (within 0.03 pH) and at the same time the transfer of oxygen is aided. That each of these processes facilitates the other was pointed out by L. J. Henderson (1921).

From the viewpoint of acid base balance it is important that a certain amount of base is in a labile condition and can be exchanged between CO_2 and Hb in respiration.

In the red blood cell this interchange may take place directly, but since neither sodium nor potassium diffuses through the cell wall, to any appreciable extent in this exchange, the exchange between cells and serum must be indirect. It is accomplished by the transfer of Cl^- and H^+ ions between cell fluid and serum fluid. Thus:

$$\underbrace{KCl + HHb + O_2 \rightleftarrows KHbO_2 + H^+ + Cl^-}_{\text{cell}} \rightleftarrows \underbrace{H^+ + Cl^- + NaHCO_3 \rightleftarrows NaCl + H_2CO_3}_{\text{serum}} \quad (8)$$

ALKALI RESERVE

The importance of the system $\frac{[H_2CO_3]}{[BHCO_3]}$, in serving as the first line of defence against abnormal acids, lies in the ease with which excess H_2CO_3 may be excreted through the lungs. As has been pointed out in discussing equation (5) the concentration of the system $\frac{[H_2CO_3]}{[BHCO_3]}$ may be changed so long as the ratio is constant, without change in pH. Thus whenever base is needed for neutralization of a stronger acid HX it can be furnished by the $BHCO_3$.

$$BHCO_3 + HX = BX + H_2CO_3 \qquad (9)$$

and the freed H_2CO_3 can be removed by increased respiration. Excess of alkali may be met by the reverse process of decreased CO_2 elimination. The $BHCO_3$ concentration in normal venous blood is maintained at between 50 and 70 vols. per cent of CO_2. The base of this $BHCO_3$ constitutes the alkali reserve against abnormal acid. The determination of the $[CO_2]$ of blood therefore serves as the most convenient means of measuring this alkali reserve. Since the amount of total CO_2 thus bound is dependent upon the pCO_2, Van Slyke and Cullen (1917a) used the CO_2 capacity (the total CO_2 content after exposure to normal pCO_2) as the measure of the alkali reserve. More recently Van Slyke (1921b) and the present authors have expressed the alkali reserve as the $BHCO_3$ content at constant pH.

CO_2 ABSORPTION CURVES

The influence of the total buffer systems of blood is most clearly shown graphically by CO_2 absorption curves. Two types of curves are generally used. One is with $[BHCO_3]$ or total $[CO_2]$ as the ordinate and pCO_2 as abscissae. Such a curve, introduced by Bohr, was used in a very convenient graph by Haggard and Henderson (1919) giving both pH and $[H_2CO_3]$ values in addition.

In such a graph the difference in $[BHCO_3]$ with change in CO_2 tension is the measure of the buffer exchange of base with change in pH.

For many purposes a graph using $[BHCO_3]$ and pH as coördinates is preferable. Such a diagram is that shown in figure 2 which is adopted from Van Slyke's (1921b) chart. We have added to Van Slyke's diagram lines indicating CO_2 tension (Cullen and Jonas, 1923).

These curves indicate at once various conditions possible in the blood acid base system. Henderson, Bock, Field and Stoddard (1924) have recently given examples of many possible combinations of curves useful in studying the blood electrolyte equilibrium.

INFLUENCE OF SALTS AND PROTEINS ON THE ACID BASE EQUILIBRIUM

The salt systems constituting the buffer systems in the blood comprise only one-fifth of the total salt concentration of the serum or blood. The total monovalent salt concentration in normal serum is from 0.15 to 0.16 N. (15–16 millimols). The *variation* in neutral salt content in the serum and tissue fluids is probably not enough to materially affect the pH of the fluids through change in the salt effect upon dissociation. Variation in the content of the proteins alters the buffer content of the blood but not significantly the various dissociation constants.

However, changes in total salt and protein concentration in serum and cell fluid cause shifts in the distribution of the ions, through the change in water content, in osmotic pressure and in the Donnan equilibrium.

The Donnan theory of membrane equilibrium states that when two solutions a and b, separated by a semi-permeable membrane, contain both diffusible and non diffusible ions, the diffusible ions distribute themselves thus:

$$\frac{[A]_a}{[A]_b} = \frac{[A']_a}{[A']_b} = \frac{[B]_b}{[B]_a} = \frac{[B']_b}{[B']_a} \tag{10}$$

Where A and A′ represent any two species of univalent anions and B and B′ any two species of univalent cations. The brackets indicate "activities", i.e., the concentrations which the substances would have in "ideal" solutions in order to give the observed hermodynamic effects, osmotic pressure, etc. Applied to the serum cell system we have:

$$\frac{[Cl^-]_s}{[Cl^-]_c} = \frac{[HCO^-_3]_s}{[HCO^-_3]_c} = \frac{[H^+]_c}{[H^+]_s} \tag{10a}$$

The combined effect of water shift and Donnan equilibrium has been discussed by Warburg (1922) and by Barcroft *et al.* (1922).

More recently Van Slyke, Wu and McLean (1923) and Henderson, Bock, Field and Stoddard (1924) have added more data and discussed extensively the question of electrolyte equilibrium as applied to blood.

RELATION BETWEEN pH OF BLOOD AND SERUM

Blood is a heterogenous system of serum and cell phases. Each of these phases, to be strictly accurate (Warburg, 1922; Van Slyke, Wu and McLean, 1923) may be subdivided into protein and water phases, but for this review we will consider blood only as divided into serum and cell phases.

Parsons (1917) pointed out that all pH measurements made on whole unhemolyzed blood were in reality measurements of pH of serum, and that all the values reported in the literature determined by electrometric methods on blood represented the pH of the serum of the reduced blood. He further showed in association with Barcroft and others (1922) that the pH of the serum of completely reduced blood is about 0.05 pH less acid than the serum of the blood completely oxygenated at the same CO_2 tension. This indicates a serum pH difference between ordinary venous and arterial blood of about 0.02 pH at the same pCO_2.

Whenever the term "pH of blood" is used it must be understood to mean the pH of the blood serum.

pH OF RED BLOOD CELLS

The blood cells are more acid than is the serum (Warburg, 1922; Van Slyke, Wu and McLean, 1923; Henderson *et al.*, 1924) by from 0.08 to 0.14 pH at normal serum pH.

Little is known of the actual change in reaction of the blood cells but it exerts an influence on electrolyte and water distribution between cells and serum. With change in anion (Cl^- and HCO^-_3) and H^+ concentration, water is shifted between cells and serum thus changing the cell volume.

RELATION BETWEEN [$BHCO_3$] OF BLOOD AND SERUM

The [$BHCO_3$] of serum is higher than that of the cells and therefore higher than the [$BHCO_3$] of whole blood. The determinations of

total [CO_2] can be made on either whole blood or serum. The relationship between the [CO_2] of serum and of whole blood is dependent upon the pH of the serum and upon the cell volume. In considering the relation between [$BHCO_3$] of serum and cells it must be remembered that Na and K ions under ordinary conditions do not diffuse between cells and serum. The shift of base with change in oxygenation and reduction of hemoglobin in the cell results (see equation (8)) in a migration of Cl^-, HCO^-_3 and H^+ through the cell wall and in the serum an exchange of base between Cl^- and HCO_3^-. This changes the relative osmotic concentration in cells and serum and involves a water shift between serum and cells. The relationship between serum and whole blood [$BHCO_3$] has been studied and reviewed recently by Warburg (1922), by Peters and his associates (1923, 1924) and by Van Slyke, McLean and Wu (1923).

Another relationship that is of interest is that between total [CO_2], the value actually determined by CO_2 analysis, and [$BHCO_3$]. The difference between these two quantities is commonly written as [H_2CO_3] in solution and can be calculated if either pCO_2 or pH is known. This relation is discussed later.

At normal pH of 7.3 to 7.4 the [$BHCO_3$] represents about 95 per cent of the total [CO_2].

In studying variation in the acid base equilibrium for clinical and physiological purposes, it is most accurate and convenient to use plasma or serum for both pH and [CO_2] since the influence of any change in cell volume is eliminated.

pH OF SERUM OR PLASMA

For most studies of acid base balance, plasma from 0.3 per cent oxalated blood, and serum may be considered interchangeable, although, as pointed out by Warburg (1922) excessive amounts of oxalate shift the electrolyte between cell and serum and Hooper, Smith, Belt and Whipple (1920) demonstrated significant changes in cell volume arising from dry oxalate used as an anticoagulant. However, plasma is perhaps preferable for colorimetric determinations (see Methods).

REGULATION OF pH OF BLOOD

The manner in which respiration, by increase or decrease of CO_2 tension serves as a mechanism by which the reaction of the blood may be maintained within its normal range, is outlined above in the discussion of the buffer system. The nervous control of this mechanism is located in a respiratory center of the brain. For many years it was thought that the CO_2 tension was the sole chemical factor that controlled respiration, then with knowledge of the constancy of the pH of the blood, and following the experiments of Hasselbalch and Lundsgaard (1912) it was generally accepted that it is the pH of the blood which acting upon the respiratory center controls respiration. More recently a different point of view has come to be held (Scott, 1918; Jacobs, 1920; Gesell, 1923; Cullen, Austin *et al.*, 1923; Cullen and Jonas, 1923; Van Slyke, Hastings, Murray and Davies, 1924). The last mentioned workers conclude

"that when the respiratory mechanism is normal increase in alkaline reserve is only partially compensated by increase in CO_2 tension so that increase in pH also occurs. In the same way decrease in alkaline reserve is accompanied by decrease in pH. There is a decrease in CO_2 tension but not sufficient to prevent pH change. The usual percentage change in hydrion concentration is about twice that in CO_2 tension. The arterial CO_2 tension is kept normally between 35 to 45 mm., which is a much narrower range than would be necessitated for the maintenance of normal pH. The conception of the CO_2 tension as a factor physiologically important only from its relationship to blood pH is not consistent with these facts. When conditions force the organism to choose between change in CO_2 tension and change in pH it tends to compromise between the two, and acts in a manner to indicate that maintenance of normal CO_2 tension is in itself an important factor."

Possibly it is the reaction of the respiratory center as distinct from that of the blood, which is the important factor in control. In addition disturbances in the nervous mechanism may change the response to chemical stimulation (J. S. Haldane, 1922). The normal physiological variation in serum between arterial and venous blood during respiration amounts to about 0.04 pH and about 7 mm. of pCO_2 (see charts No. 116 and No. 119 of Henderson *et al.* (1924)).

CHAPTER II

Normal pH Values

pH of normal serum and plasma

The range of normal variation in serum pH was given by Van Slyke (1921b) as pH 7.3 to 7.5. Cullen and Robinson (1923) studying normal students with the colorimetric method found a variation from 7.28 to 7.41. All but two, 7.28 and 7.41 fell within the limits 7.40 to 7.30.

Bigwood (1923) with another series with the same method confirms this variation of 7.30 to 7.40. Myers and Booher (1924), using the same dilution but reading the determination in Myers colorimeter found the limits of normal pH to lie between 7.35 and 7.43. However, Koehler (1923) and Marrack and Boone (1923) found somewhat higher normal pH values as do Hastings, Neill, Morgan and Binger (1924) in arterial blood. The values of Chambers and Kleinschmidt (1923) are much lower and must represent a different pH standardization. We have taken for this review the normal outline indicated in figure 1 with pH range from 7.30 to 7.50.

In the observations of Cullen and Robinson the zone 7.30 to 7.40 was found normal not only for various individuals but for a single individual. That is, the pH of any individual might on successive days vary between 7.30 and 7.40.

Drucker and Cullen have developed a technique for taking capillary blood from the heel of infants or the ear of adults without loss of CO_2, using Hawkin's modification of Cullen's method for pH and employing 0.25 cc. of blood. They find the pH of normal infants' capillary blood to lie between 7.34 and 7.44. Since the capillary blood has been shown by Lundsgaard to approximate arterial blood in composition its pH will be about 0.03 more alkaline than venous blood. The range observed, therefore, is similar to that found by Cullen and Robinson for adults.

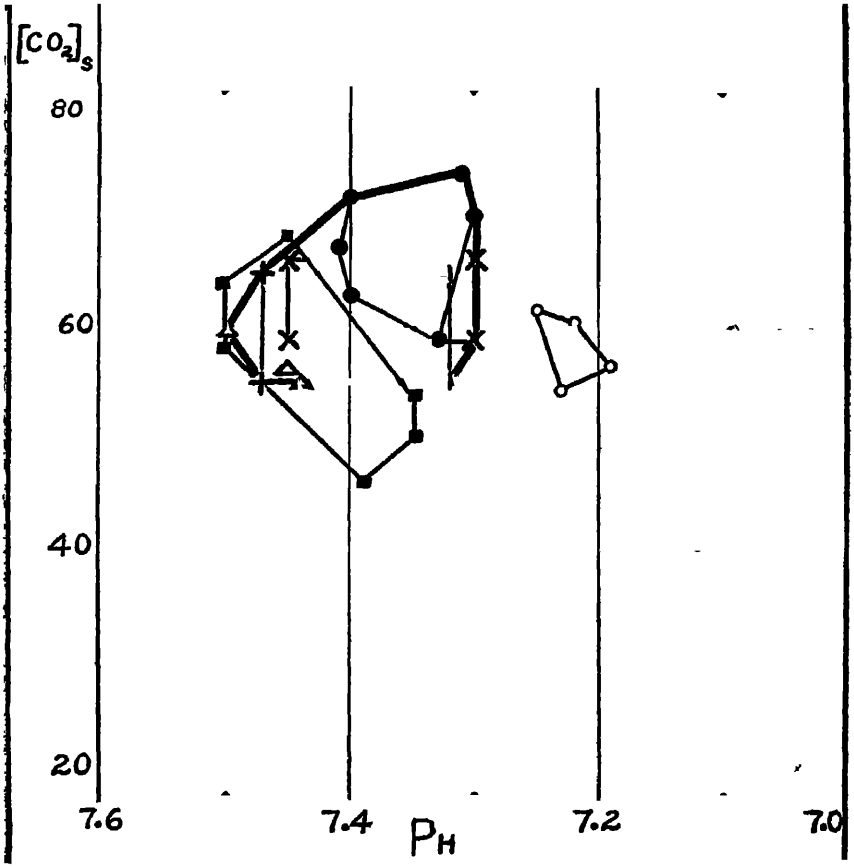

FIG. 1. NORMAL PLASMA [CO_2] AND pH VALUES

• • Cullen and Robinson (1923).

x——x Marrack and Boone (1923).

+——+ Myers and Booher (1924).

Δ Koehler (1923).

■——■ Hastings, Neill, Morgan and Binger (1924); [CO_2]s values raised from blood to serum values but not corrected from arterial to venous values.

o——o Chambers and Kleinschmidt (1923).

Values taken as normal for purposes of this review.

pH OF BODY FLUIDS OTHER THAN SERUM

As stated by Van Slyke (1921b) our limited knowledge of body fluids indicates that extracellular fluids have approximately the same pH as serum. Parsons and Shearer (1920) report that the pH of cerebrospinal fluid is that of normal plasma pH. Boots and Cullen (1922) found in nonpurulent joint exudate in rheumatic fever pH values of 7.3 to 7.4. In this laboratory we observed the same pH for edema fluid and blood serum.

pH of purulent fluids. Whenever the pH of purulent fluids has been measured it has been found more acid than the serum. In purulent joint exudates Boots and Cullen (1922) found pH of 6.40. Lord has reported pH of 6.8 to 6.2 in consolidated portions of lungs in lobar pneumonia. This range has also been observed by Avery and Cullen (unpublished).

Intracellular pH. Little is known of intracellular reaction other than that of the red cells which is estimated by Warburg (1922) and by Van Slyke, Wu and McLean (1923) for normal serum pH of 7.4 to be 0 08 to 0.14 pH more acid than serum. Probably other intracellular fluids vary, at least as much from that of serum.

pH of secretions. It is interesting that the cells of the body although bathed with fluid of such constancy of pH as serum, can function in contact with a very high degree of acidity. The pH of gastric juice may be less than 2. The pH of urine ranges from 5.0 to 8.5; duodenal fluid may have a pH over 8.0. Saliva has a normal pH of 6.7 (Starr, 1922).

CHAPTER III

pH of Plasma in Disease

ABNORMAL ACID BASE BALANCE

The terms *acidosis* and *alkalosis*, common in clinical usage, are often used by different individuals to describe entirely different conditions. Because the first recognition of abnormal acid base balance came through the knowledge that in diabetes abnormal metabolism resulted in the excretion of aceto-acetic acid, β-oxybutyric acid and acetone, the term *acidosis* is often used to describe either a ketonuria or a ketonemia. The term *acidosis* has been applied to the following conditions, (1) acetone bodies in the urine (2) acetone bodies in the blood, (3) decreased alkali reserve, (4) decreased CO_2 tension of alveolar air, (5) and decreased pH of plasma.

This confusion is well recognized in physiological work and various attempts have been made to establish a more precise terminology. Van Slyke (1921b) in his review of abnormal and normal acid base balance divided the possible conditions of abnormality into eight groups which are best visualized from his diagram which is used with slight relocation of the normal area as the basis of figure 2.

The Report on Acidosis of the British National Research Council, proposes the terms *alkalosis* and *acidosis* to indicate high and low levels of [$BHCO_3$] and *alkalemia* and *acidemia* to indicate high and low pH. Hasselbalch, Haldane, Van Slyke and the present authors (in studying the acidosis of anesthesia) have used "*true acidosis*" to mean coincident abnormally low pH and [$BHCO_3$].

It is probable, however, that no general usage for the term *acidosis* can be agreed upon and it is therefore better to avoid the term wherever possible or else to state the exact sense in which it is used.

For the present it is best to state the conditions of the acid base balance in terms of two of its factors; either in terms of Van Slyke's nine areas, or perhaps simply as an acid base condition of 7.4 and

50, indicating $pH_s = 7.4$ and $[CO_2]_s = 50$ volumes per cent. The term "A.B.C. (acid-base condition) of 7.4 and 50" might supply the need for an easily handled term. The nature of the coördinates 7.4 and 50 make confusion between them impossible. Whatever the terminology, it is evident that determination of only one of the three variables pCO_2, pH or $[CO_2]$ is not sufficient to determine the acid base condition.

The studies of the alkali reserve of the blood in disease are much more numerous than those dealing with direct measurement of the pH of the plasma. Simultaneous studies of CO_2 tension and CO_2 content of the plasma in disease from which the pH can be calculated are also rare.

Accumulation of acids other than HCO_3^- in the blood with depletion of the blood bicarbonate has been described as a rule as occurring at first without change in pH and only after considerable reduction in the bicarbonate to be associated with a more acid serum pH. Such a course of events is approached in the acidosis of fasting, diabetes and nephritis in figure 2. The compensated stage of alkali deficit was believed to be brought about through a response of the respiratory center to an unmeasurably small diminution in pH with consequent stimulation of pulmonary ventilation, and consequent diminution in pCO_2 in alveolar air and arterial blood just sufficient to secure an almost proportionate reduction of bicarbonate and of CO_2 tension with almost constant pH.

Only when the respiratory mechanism fails to function adequately and to compensate was change in pH believed to occur. With the introduction of Cullen's (1922) method for direct measurement of the pH of serum or plasma as drawn and the application of this method to disease we are recognizing, however, that fall in plasma bicarbonate due to introduction of other acids, is often associated throughout its course with rise and fall of pH as indicated by the numbered arrows in figure 2. Evidence for this is to be found in the studies we describe of acid and alkali administration, of anesthesia, of exercise and radiation. This behavior of blood reaction has a bearing upon the studies of the regulation of the respiratory center and the relative importance of pH and of pCO_2 in this regulation. This has been previously discussed.

EXCESSIVE INTRODUCTION OF ACIDS

Many experimental studies have been made upon the effects of acid fed by mouth or administered intravenously. The effect, we have observed in the dog, from injecting HCl, H_3PO_4, NaH_2PO_4 and lactic acid is shown in table 1. These injections were made under local anesthesia into the vein (in one case into the heart) using M HCl, M/10 H_3PO_4, M/10 NaH_2PO_4 and M/1 lactic acid.

The changes in pH and [$BHCO_3$] are shown in table 1. The direction and magnitude of the changes in the acid base equilibrium are shown by the numbered arrows in figure 2.

The apparently less marked effect of H_3PO_4 on the [$BHCO_3$] and its greater effect on pH is due to the large amount of acid injected leading to almost complete disappearance of $BHCO_3$ from the blood. The

TABLE 1

ACID	CONCENTRATION	AMOUNT INJECTED PER KILO	TIME OF INJECTION	Δ[$BHCO_3$] PER MILLIMOLE OF ACID PER KILO	Δ[pH] PER MILLIMOLE OF ACID PER KILO
	M	*mM.*	*minutes*	*mM.*	
HCl	1.0	2.2	10	−6.0	−0.11
HCl	1 0	2.2	10	−7.0	−0.06
HCl	1 0	1.4	6	−6.0	−0.06
H_3PO_4	0 1	4 9	49	−4 0	−0.16
NaH_2PO_4	0 1	4 9	52	−2.0	−0.03
Lactic	1 0	2.2	6	−2.0	+0 01

less pronounced effect of NaH_2PO_4 both on bicarbonate and pH is to be expected. The absence of effect of lactic acid on pH is, however, interesting. At the pH of blood the ratio of $\frac{\text{lactic acid}}{\text{sodium lactate}}$ is about $\frac{1}{3200}$, taking the dissociation constant of lactic acid as 1.38×10^{-4} (Landolt-Börnstein 4th edition p. 1147). The markedly diminished effect of lactic acid per millimole on the bicarbonate as compared with HCl is therefore not to be expected except as a result of disappearance, of the lactic acid, as for instance, by synthesis to glucose or as a result of its wider distribution in the body fluids as in the case of bicarbonate, later described.

When HCl is given by mouth the change in plasma bicarbonate and pH depends on the relative rate of absorption and of compensating excretion. In the observation of Gamble and Ross (1923) the administration to a child of 17 mM. per kilo of HCl in 2 days lowered the plasma bicarbonate 9 mM. and the pH 0.17. Haldane (1923) and Gamble and Ross (1923) pointed out that the ingestion of ammonium chloride in man causes marked and prolonged acidosis, due presumably to conversion of part of the ammonia into urea, thus freeing the acid which had been combined with it. The acidosis is shown by fall in the plasma CO_2 capacity and fall in the alveolar pCO_2. There is a roughly commensurate increase in the rate of acid plus ammonia excretion in the urine, the ratio between the increase in acid and increase in ammonia being dependent upon the phosphates available for excretion. As acidosis continues the phosphate excretion falls, and at this time a glucose tolerance test produces greater rises in blood sugar. The fall in plasma bicarbonate is replaced almost molecule for molecule by chloride in Haldane's studies.

$CaCl_2$ ingested causes similar change in the plasma bicarbonate and chlorides as shown by Gamble, Ross and Tisdall (1923). The Ca^{++} is mainly excreted as $CaCO_3$ in the feces and the Cl^- is absorbed, replacing HCO_3^- and causing true acidosis with low pH and low $[BHCO_3]$.

Both NH_4Cl and $CaCl_2$ are powerful diuretics. Haldane produced a fall in weight of 7 pounds in three days after 65 grams of NH_4Cl and a rise in hemoglobin of 20 per cent even although he drank water freely; and Levy noted a similar increase in serum protein after $CaCl_2$ ingestion. During NH_4Cl ingestion Haldane observed the following urinary excretions expressed in percentage of the normal values: Na 250 per cent, K 520 per cent, Ca 330 per cent, P 180 per cent. The diuresis and the salt excretion are attributed by Haldane to the fact that the colloids of the body, being brought nearer their isoelectric point by the fall in pH, retain less water and base.

The serum Ca has been found by Haldane (1924) to increase 10 per cent following NH_4Cl ingestion. $CaCl_2$ ingestion can cause an increase of 25 per cent in serum Ca. Sodium bicarbonate conversely lowers the serum Ca 10 to 20 per cent. Ammonium chloride has been used in the treatment of tetany by Freudenberg and Gyorgy and in

lead poisoning by Aub and his associates with striking effect. In infantile tetany the symptoms vanish in a few hours and satisfactory results have also been obtained in gastric tetany and post operative tetany. In a series of infants treated with 0.5 gram per kilo per day of ammonium chloride the serum calcium rose from a mean value of 6.6 mgm. per 100 cc. to 8.9, the phosphorus falling from 4.9 mgm. per 100 cc. to 2.9. Gollwitzer–Meier (1924) has reported a fall in plasma bicarbonate after intravenous ingestion of $MgCl_2$ in rabbits.

EXCESSIVE INTRODUCTION OF BASE

Introduction of base into the body increases the bicarbonate of the blood and the pH. Oral bicarbonate administration in human beings has been found by Palmer and Van Slyke (1917) to raise the bicarbonate of the blood according to the assumption that the body contains 70 per cent of fluid and the bicarbonate is distributed throughout this fluid. According to this assumption the following equation was calculated. Increase in plasma CO_2 in volumes per cent $= \frac{38g}{W}$, g being grams of sodium bicarbonate administered and W the body weight in kilos. Or this may be stated that for each mM of bicarbonate administered per kilo, the increment in serum bicarbonate is 1.43 mM. This indicates a much wider distribution of the orally administered bicarbonate than of injected HCl or H_3PO_4 in our experiments described above. In our experiments with intravenously injected HCl the effect is as if distributed in 1/6 or 1/7 of the body weight instead of in 70 per cent of the body weight. The direction of the change in the acid base equilibrium following alkali administration is shown in figure 2.

EXCESSIVE LOSS OF ACID FROM THE BODY

Loss of HCl from the body such as occurs in pyloric obstruction also gives rise to increase in plasma bicarbonate (MacCallum *et al.* 1920; Hastings, Murray and Murray, 1921; Grant, 1922), although in the experimental studies of Hastings, Murray and Murray the rise in bicarbonate was not associated with any significant rise in pH.

RENAL DISEASE

Since the elimination of acid from the body is chiefly by CO_2 elimination through the lungs and by excretion of other acids as acid phos-

phates and combined with ammonia through the kidneys, it might be expected that impaired renal function would induce acidosis. Nephritis and other forms of renal disease associated with impairment of renal function do as a matter of fact give rise to disturbance of acid base equilibrium. Wallace and Pellini (1921) point out, however, that double nephrectomy does not produce acidosis in animals and therefore attribute the acidosis of nephritis to some other factor than mere impairment of acid excretion. Lepine (1879) and von Jaksch (1888) by titration of the ash of blood demonstrated a diminished alkalinity in the blood of uremics. Straub and Schlayer (1912) measured the CO_2 tension of the alveolar air in eight cases of uremia and found in the patient with highest values 34 to 39 mm. pCO_2 and in the patient with the lowest, 11 to 17 mm. On the basis of this and the hyperpnea of uremics they suggested that acid intoxication is a factor in uremia. Von Hoesslin (1912) pointed out the influence of alkali administration in diminishing the albuminuria and cylindruria in some cases of nephritis and called attention to the difference in amount of alkali necessary to change the reaction of the urine in different cases. Sellards (1912) introduced the measurement of the amount of $NaHCO_3$ necessary by mouth to render the urine alkaline as a test for the detection of acidosis and found that whereas 5 grams by mouth suffices in the normal individual, this dose was effective in only one out of nine patients with chronic diffuse nephritis and two patients tolerated 60 and 130 grams respectively intravenously and still excreted acid urine. Palmer and Van Slyke (1917) showed that the depletion of the alkali reserve could be better gauged by noting the amount of alkali that must be administered to cause excretion of a *less acid* urine rather than of an *alkaline* urine.

Peabody (1914, 1915) showed that as chronic diffuse nephritis increases in severity as measured by impairment of phthalein excretion and retention of non-protein nitrogen in the blood there appears at first little or no evidence of acidosis, then an increase in Sellard's tolerance to alkali and finally in advanced cases, verging on uremia, a diminution in the alveolar CO_2 tension which in the terminal stages of uremia may be very marked. He noted there was not a strict parallelism between the other evidences of impairment in renal function and the degree of acidosis but only a general tendency for both to become

marked in advanced cases. The dyspnea of cardiorenal disease also was commonly more marked than could be accounted for merely by the acidosis. Lewis and his coworkers (1913), and Palmer and Henderson (1913) came to substantially these conclusions. Chace and Myers (1920) using the method of Van Slyke and Cullen (1917) for the CO_2 capacity of the venous plasma similarly demonstrated this acidosis. Howland and Marriott (1916) demonstrated an increase in the phosphate of the serum in nephritis with lowered CO_2 capacity of the plasma and with decreased plasma pH by the dialysis method of Levy, Rowntree and Marriott. Greenwald (1915), Feigl (1917a, 1917b), Denis and Minot (1920), Salvesen and Linder (1923) have also shown retention of acid phosphate in nephritics. Means and Rogers (1917) reported an extreme acidosis in a man with bilateral polycystic kidneys complicated by a septic infection of the hand. Two days before death he had extreme hyperpnea with a ventilation of 51 liters per minute, an alveolar pCO_2 (Plesch) of 6.4 mm., a blood urea of 332 mgm. per 100 cc. (55 mM.), a CO_2 capacity of the plasma of 12 vols. per cent (5.4 mM.), no phthalein excretion in three hours, serum phosphorus of 18 mgm. per 100 cc. (5.8 mM.) serum calcium 3 mgm. per 100 cc. (0.75 mM.). At this time 110 grams $NaHCO_3$ by mouth failed to render the urine alkaline.

If one compares the retention of phosphate with the diminution of bicarbonate in the serum it would seem that the acidosis can not always be explained simply by the phosphate retention however. In Means and Rogers case with a rise of [P] of 4.3 mM. representing about 8 meq. of base above the upper normal, there is a fall of [$BHCO_3$] of 13 mM. below the lower normal Some other factor appears to be concerned here than merely phosphate retention. The data of Salvesen and Linder (1923) show very little change in the serum base in nephritics beyond the normal limits which makes it probable that acid other than HCO_3^- is increased.

MacNider (1920) has demonstrated disturbances in the acid base equilibrium in experimental nephritis in animals and here the acidosis is possibly even more conspicuous than in clinical nephritis. This author and also Nagayama (1920) and others have demonstrated improvement in renal function when the disturbance in acid base equilibrium is corrected by alkali therapy and MacNider has demon-

strated a diminished susceptibility of the kidney to injury by toxic substances when by alkali therapy the acidosis is prevented.

While these studies indicate the depletion of alkali reserve in nephritis, direct studies of the pH of the blood as it exists in vivo such as are furnished by the method of Cullen (1922) are at present available in the literature for only a few cases. Cullen and Jonas (1923) cite one case of uremia with plasma pH 6.7 and [CO_2] of 4 vols. per cent. Cullen and Stillman at the Rockefeller Institute found a pH of 7.12 with a low normal [CO_2] of 50 vols. per cent in the plasma of a patient with acute convulsions and unconscious but without uremia. Strenuous alkali therapy brought the pH to 7.35 and the [CO_2] to a high normal and the patient recovered from the attack. This case is a striking example of the value of pH studies. Linder, Hiller and Van Slyke (1925) report 17 observations on 10 patients with various types of nephritis. One case of nephrosclerosis and one of chronic nephrosis showed high normal bicarbonate with normal pH. In those forms of glomerulonephritis associated with marked retention of urea there is a tendency to depression of both bicarbonate and pH; the lowest observation being [$BHCO_3$] = 27.8 volumes per cent with pH = 7.16. The zone occupied by these cases is shown in figure 2. Myers and Booher (1924) report 15 cases of nephritis among their 64 cases of abnormal acid base balance, seven of these with less marked depression of [CO_2] showed pH within the lower limit of normal (Hasselbalch's and Van Slyke's compensated acidosis) while eight cases showed abnormally low pH and more marked depression of [CO_2] (uncompensated or true acidosis). However, if the observations on the 15 cases be plotted as in figure 2, the distinction between the compensated and uncompensated groups is seen to be largely an artificial one, due to the width of the normal pH zone. The evidence is that any lowering of [CO_2] tends to be associated with more acid pH.

CARDIAC DISEASE

In cardiac disease uncomplicated by impairment of renal function Peabody (1914) found no evidence of alteration of pH nor of acidosis that could account for hyperpnea. Wilson, Levine and Edgar (1919) found normal CO_2 capacity of the plasma in cases of "irritable heart."

DIABETES MELLITUS

Diabetes mellitus because of the accumulation of abnormal products of metabolism has long been the classic example of disturbance of acid base balance. Of the older literature in regard to presence of acetone bodies there are many reviews. The relation of alkali reserve to alveolar CO_2 and to titratable alkali was shown by Stillman, Van Slyke, Cullen and Fitz (1917). It has long been recognized that low alveolar CO_2 tension is an index of low alkali reserve. If the low [$BHCO_3$] is accompanied by sufficient decrease in pCO_2 to prevent change in pH, the condition is that of "compensated acidosis." (Hasselbalch; Area 6, Van Slyke). It was also recognized that coma of diabetes is associated with a true acidosis in the sense of decreased pH and decreased [$BHCO_3$].

Before the discovery of insulin diabetic coma was only occasionally strikingly benefited by alkali treatment.

One of the most impressive demonstrations of the action of insulin is afforded by the changes in the acid base balance in diabetic acidosis following insulin treatment. Chart No. 3, p. 547, Cullen and Jonas (1923) shows this most clearly. A patient with a plasma pH of 6.98 and [$BHCO_3$] of 16 vols. per cent had his plasma restored in one day to a normal range with a plasma pH of 7.32 and [$BHCO_3$] of 41.5 vols. per cent. The insulin altered the metabolism so that not only did further accumulation of acid bodies cease but the acetone bodies already combined with the base were oxidized thus freeing the base to recombine with CO_2.

This chart and figure 2 in the present review also show other interesting facts concerning insulin. With excess of insulin the [$BHCO_3$] and pH tend to become abnormally high. Such pH studies in stupor from excess of insulin are very few but there are indications in the experiments of Cullen and Jonas that it tends to be associated with an alkalosis (high pH and high CO_2).

One of their patients upon breaking his diet immediately started back toward his initial acidosis but this was easily checked with insulin.

In the data of Cullen and Jonas a tendency to constancy of the pCO_2 rather than of pH is exhibited by a number of individuals, but not in all their cases.

These results are in entire agreement with those reported simultaneously by Bock, Field and Adair (1923) and since by Myers and Booher (1924). It would appear that insulin treatment alone, without alkali is sufficient to restore the acid base balance from diabetic acidosis to a normal state or to one of alkalosis. It is also evident that the human organism can recover after reduction of the serum pH to as low a figure as 7.0.

RHEUMATIC FEVER

Because of the old theory that the joint fluid of rheumatic fever was sufficiently acid to liberate free salicylic acid, Boots and Cullen (1922) studied electrometrically and colorimetrically the pH of the joint fluids from such cases. Sterile joint fluids showed a pH approximately that of normal blood. In a few of these cases the plasma pH was determined and found to be within normal limits (unpublished results). There is therefore no evidence of a disturbance of the acid base equilibrium in rheumatic fever nor of the possibility of the mechanism of salicylate action mentioned above.

ACIDOSIS OF CHILDREN

Howland's and Marriott's (1916) studies indicated that an acidosis is associated with the dehydration of infants. Mitchell and Jonas, (1925) found that although low pH and low [CO_2], i.e., a true acidosis, might be associated with such conditions, such was not invariably present. Further there was no constant relationship between the severity of the disease and the acidosis. They conclude that the acidosis when it occurs is a result of the general derangement and not a causative factor in the condition.

FASTING

Gamble, Ross and Tisdall (1923) have studied the behavior of the acid base equilibrium during fasting in four epileptic children, with reference to the factors regulating excretion of acid and base. Their studies deal also with the changes in alkali reserve of the serum but do not furnish data concerning the pH.

Bigwood (1924b) and Bigwood and Geyelin (in press) have studied the pH and [CO_2] of plasma during the fasting treatment for epilepsy.

Koehler (1923) found after fasting 50 hours a mean depression of 5 vols. per cent [CO_2] and of 0.02 in pH. After 77 hours fasting the mean depression in [CO_2] was 8 vols. per cent and in pH a fall of 0.10.

ANOXEMIA

The influence of anoxemia in inducing acidosis has been a subject of much theorizing. Wallace and Pellini (1921) studied a variety of toxic substances as to their effect upon the alkali reserve. They found that uranium, cantharidin, diphtheria toxin, large doses of arsenic, sodium nitrite when given in a dose large enough to cause methemoglobin formation, and potassium cyanide all produced marked fall in the plasma bicarbonate whereas emetin hydrochloride, hydrazin and morphin produced no fall of alkali reserve. The first four substances mentioned are capillary poisons. An extremity poisoned with diphtheria toxin when transfused with normal arterial blood returned the venous blood with a lowered alkali reserve and this led the authors to the belief that the acidosis arose from a disturbance of the muscle metabolism. The greater fall in alkali reserve from the first four poisons than following double nephrectomy led them to exclude mere impairment of renal excretion as the important factor in the acidosis. Poisons which like emetin and podophyllin are selective for the intestinal capillaries failed to produce any marked acidosis; nor did marked liver injury from hydrazin cause acidosis. They concluded that after large doses of sodium nitrite with methemoglobin formation, after cyanide which stops tissue oxidation and after the first four poisons mentioned above, which are general capillary poisons, the acidosis results from interference with tissue oxidation and they emphasize the importance of capillary poisons as a cause of acidosis.

This conclusion with regard to cyanide poisoning is confirmed by the work of Holboell (1924), who found that the alkali reserve and pH are reduced in cyanide poisoning and that the venous blood is returned as fully oxygenated as the arterial blood, indicating that the interference with oxygen utilization is in the tissues.

pH, LACTIC ACID AND KETONURIA

Davies, Haldane and Kennaway (1920) have called attention to the fact that when either from hyper-ventilation of the lungs and con-

sequent decrease in the pCO_2, the pH of the blood is increased, or when from bicarbonate ingestion the alkali reserve is increased with, or possibly without, increase in the pH of the blood, there is a tendency for acetone bodies to appear in the urine. This ketonuria, the mechanism of which is not clear, but occurring during an alkalosis, is evidence of the importance of distinguishing between ketonuria and the state of the acid base balance in the blood. Macleod and his co-workers (1917, 1918, 1921) have shown that administration of alkali causes an increase of lactic acid in the blood and urine, and an increase of glycolysis in the blood with a decrease in blood sugar concentration. Anrep and Cannan (1923) using a Starling heart-lung preparation and defibrinated blood found that the lactic acid concentration of the blood rose and fell with the pH of the blood and concluded that the pH of the blood regulates the rate of removal of lactic acid. They suggest that this constitutes another factor in addition to the hemoglobin and other proteins and to the bicarbonate and to the phosphates of the blood, in regulating the acid base balance of the blood.

The formation of lactic acid during muscle contraction, a complete review of which is given by Hill (1922), has been demonstrated and studied quantitatively especially by Meyerhof and by Hill. The extent to which lactic acid reduces (fig. 2) alkali reserve and plasma pH during violent exercise has been shown by Barr and his associates (1923) and by Himwich and Barr (1923). The importance of oxygen in the removal of this lactic acid has also been shown by Meyerhof and by Hill but the extent to which disturbance of this mechanism occurs in disease has not been studied. The influence of pH on the recovery process of muscle has been studied by Hartree and Hill (1923, 1924). The direction and possible extent of the disturbance of the acid base equilibrium following exercise is shown in figure 2 from the data of Barr and his associates, and its magnitude is very striking.

ANAPHYLACTIC SHOCK

It is interesting to consider the evidence for acidosis in anaphylactic shock. Eggstein (1921a, 1921b), Underhill and Ringer (1921), Hirsch and Williams (1922), Bigwood, Cogniaux and Collard (1924) and Bigwood (1924a) have demonstrated a fall in alkali reserve in the

dog and in man during anaphylactic shock and in the condition of low blood pressure following the injection of protein split products, typhoid vaccines and histamine hydrochloride and Hirsch and Williams and Bigwood and his associates find also a marked fall in pH. These authors have observed some parallelism between the degree of shock or fall in blood pressure and the extent of the acidosis. Eggstein found that a fall in the CO_2 capacity of the plasma determined by the technique of Van Slyke and Cullen (1917) to below 25 vols. per cent in his experiments was usually associated with a fatal outcome and that a preliminary administration of sodium bicarbonate before the production of anaphylactic shock diminished the acidosis and lessened the mortality. Bigwood and his associates point out that the fall in $[BHCO_3]$ and in pH involves an increase in the maximal $[Ca^{++}]$ (see our discussion of tetany); a fact possibly of importance in view of Hamburger's evidence for decreased permeability of the capillary wall resulting from increase in $[Ca^{++}]$. The slope of the plotted data of Bigwood and his associates indicates a change in acid base equilibrium quite similar to that which we have observed from anesthesia. That is, associated with a true acidosis there is somewhat further depression of pH suggesting a greater depression of the respiratory center than occurs simply from administration of acid, or from nephritic or diabetic acidosis.

TRAUMATIC SHOCK

Numerous studies of the alkali reserve in traumatic shock have appeared in the literature which substantially support the findings of Cannon (1918) that there is a lowering of the alkali reserve when there is lowering of blood pressure from shock or from hemorrhage, but his conclusions as to the importance of the acidosis as a cause of the shock have not been established. Raymund (1920) has pointed out that the evdences of shock may precede the fall in alkali reserve. In five dogs traumatized under local anesthesia no striking fall in the alkali reserve appeared until the condition of the animals became quite serious. When general anesthesia is given the effects of the anesthesia upon the acid base equilibrium render the interpretation of the changes due to shock per se very difficult. (See later discussion of anesthesia.)

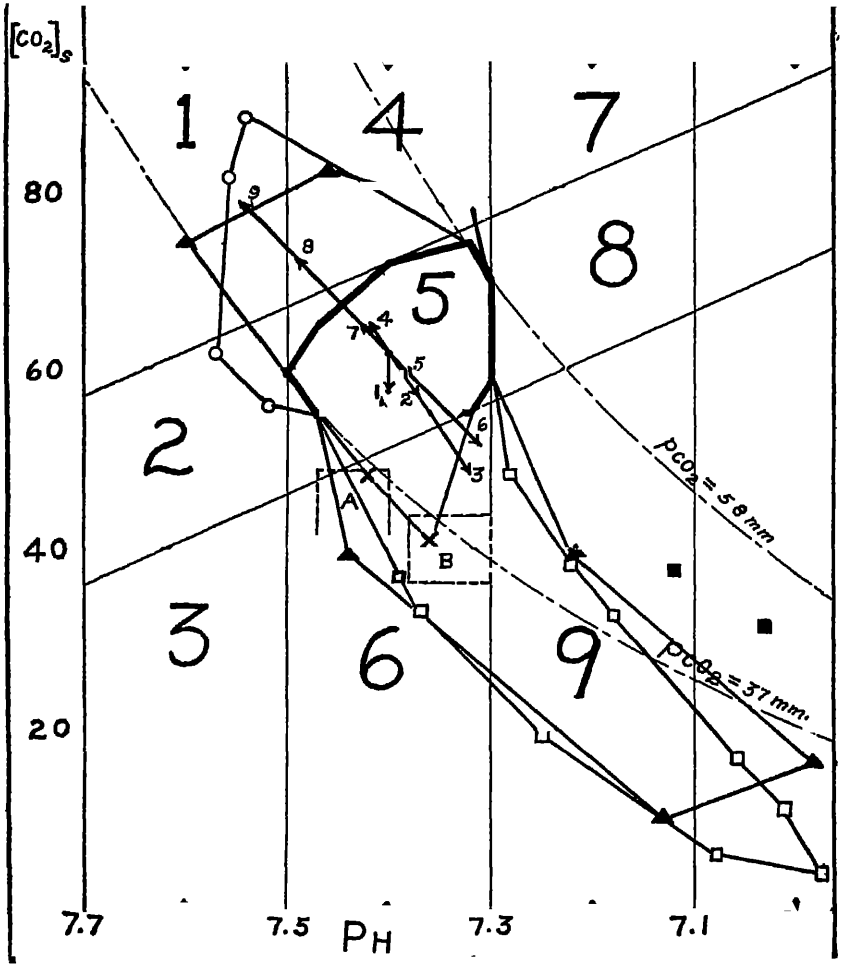

FIG. 2. VAN SLYKE TYPE OF GRAPH SHOWING NORMAL AREA OF FIGURE 1 WITH ITS LIMITING pCO_2 CURVES AND ITS LIMITING CO_2 ABSORPTION CURVES

Large figures are Van Slyke's areas

1: Uncompensated alkali excess.
2, 3: Uncompensated CO_2 deficit.
4: Compensated alkali or CO_2 excess.
5: Normal balance.
6: Compensated alkali or CO_2 deficit
7, 8: Uncompensated CO_2 excess
9: Uncompensated alkali deficit

Numbered arrows indicate direction and magnitude of change induced by

1. Lactic acid (see table 1), 2 NaH_2PO_4 (table 1); 3 HCl (table 1), 4. Ingestion of 84 mgm (1 mM) per kilo of $NaHCO_3$, 5 to 6, immediate effect of one hour of radiation (Kroetz, 1924), 7 to 8, day following radiation (Kroetz), 9 One hour of radiation (Hussey, 1922).

o———o Area including cases of alkali administration or loss of acid by vomiting (Myers and Booher, 1924).

▲ ▲ Area including diabetics with acidosis (low $[CO_2]$) and under insulin treatment (high $[CO_2]$) (Cullen and Jonas, 1923, Myers and Booher, 1924)

A Fasting 50 hours (Koehler, 1923) B Fasting 77 hours (Koehler, 1923)

□—□ Nephritis (Meyers and Booher, 1924, Linder, Hiller and Van Slyke, 1925)

■ Effect of exercise (Barr, Himwich and Green, 1923a)

——— Normal area.

TOXIC SUBSTANCES

The administration of methyl alcohol to dogs was shown by Pohl to be followed by greatly increased excretion of formic acid in the urine, the maximum excretion being reached on the third or fourth day. Krol demonstrated a well defined increase in the ammonia and creatin of the urine in dogs in similar experiments. Bongers gave methyl alcohol to dogs and measured the amounts recovered by gastric lavage repeated over several days. He asserts that he recovered about three times as much methyl alcohol in the combined washings in the second and third days as he was able to obtain in those of the first, suggesting very slow metabolism of the alcohol. In spite of this evidence of abnormal acid excretion Loewy and Munzer (1923b) found in rabbits no diminution in the blood bicarbonate Harrop and Benedict (1920) however in a human case of methyl alcohol poisoning found increase of organic acid in the urine and specifically of lactic and formic acids and in addition a fall of plasma bicarbonate to 36 vols. per cent. Alkali therapy raised the latter to 86 vols. per cent. pH studies are not available. Haskell, Hileman and Gardner (1921) found a reduction in the CO_2 capacity of most of their dogs poisoned with methyl alcohol but not in all, nor was the reduction in CO_2 capacity commensurate with the severity of symptoms. Rabinovitch (1922) found a fall in [CO_2] in one human case to 26 vols. per cent with a rise in blood phosphorus to 11.2 mgm. [P] per 100 cc.

It will be seen from figure 2 that the effects of acid or acid producing salts, of fasting, of diabetic acidosis, of nephritic acidosis, of severe exercise and probably of anaphylactic shock is to produce change in the acid base equilibrium in a similar direction and to a degree that is dependent upon the severity of the disturbance.

TETANY

Tetany is a condition intimately related to the acid base equilibrium of the blood. The studies of the pathogenesis of this condition have been recently reviewed by MacCallum (1924) and any extensive repetition of the data there so well presented seems unnecessary. Certain aspects of the condition especially in relation to the pH of the serum may be discussed further here to advantage however.

It is evident from a study of the literature that tetany is associated with one or more of the following:

a. Destruction, removal or disease of the parathyroid glands
b. Increased alkalinity of the serum
c. Diminished [Ca] and increased [P] of the serum

Binger (1917) showed that diminution in the [Ca] and increase of the [P] of the serum produced by injection of phosphate gave rise to marked tetany when the pH of the injected solution was about 6.1 or greater, gave rise to only slight tetany when the pH of the solution injected was 5.8 and to none when its pH was 4.5. With the availability of Cullen's (1922) method for direct measurement of the serum pH the authors in conjunction with H. C. Gram and H. W. Robinson carried out

TABLE 2

SOLUTION INJECTED	TIME OF BLEEDING	pH	[$BHCO_3$]	CALCULATED pCO_2	PROTEIN	[Ca] PER 100 CC.	[P] PER 100 CC.	[Cl]
			mM.	*mm.*	*grams per cent*	*mgm.*	*mgm*	*mM*
a. Na_2HPO_4 $\frac{M}{10}$	Before	7 34	19 3	40	7 1	11 1	2 0	111
	After	7.36	20.4	43	5.4	6 6	27.7	95
b. NaH_2PO_4 $\frac{M}{10}$.... ...	Before	7 28	18 9	39	8.1	10.8	4.4	111
	After	7.13	9 6	28	6.7	7 0	66.2	99
c. NaH_2PO_4 $\frac{M}{10}$, NaCl $\frac{M}{10}$	Before	7.35	20 1	39	6 7	10 4	3 5	111
	After	7 08	11 2	41	4 3	6 3	49 6	113

similar experiments thus far unpublished. Dogs were injected intravenously with (a) M/10 Na_2HPO_4, (b) M/10 NaH_2PO_4, (c) M/10 NaH_2PO_4 + M/10 NaCl. Each dog received 49 cc. per kilo in 53 minutes. Blood was taken from the left ventricle before and two minutes after completing the injection, defibrinated under oil and the serum removed without loss of CO_2. Serum analyses are given in table 2.

The methods used are the same as those employed in studies of ether anesthesia by Austin, Cullen, Gram and Robinson (1924): [Ca] and [P] were analyzed by the methods of Tisdall (1923) and Tisdall (1922b) respectively.

In the experiments quoted typical tetany appeared in experiment (a); no tetany was present in (b) and (c). It is evident that the greater tendency to tetany in (a) as compared with (b) is not due merely to the larger amount of Na administered in (a) for in (c) where the Na administered is the same no tetany developed. The difference in tendency to produce tetany in these as in Binger's experiments lies apparently with the resulting pH of the serum or with the pH and bicarbonate concentration of the serum.

Recognition of a relationship of this sort in connection with the physiological activity of Ca together with the recognized limited solubility of Ca led Rona and Takahashi (1913) to attribute the physiological activity of calcium to the calcium ion concentration and, assuming the blood saturated with $CaCO_3$, to calculate the calcium ion concentration according to the equation:

$$[Ca^{++}] = K \frac{[HCO_3^-]}{[H^+]} \tag{11}$$

Brinkman has included the phosphate ions in a similar equation on the assumption that the blood is also saturated with calcium phosphate. The most carefully developed form of this double equation is that of Kugelmass and Shohl (1924), who have evaluated the constants for these and related equations in aqueous solutions at 38°C. A form of the combined equation given by them is:

$$[Ca^{++}] = \sqrt{\frac{(7.6 \times 10^{-8})\ [H^+]}{[HPO_4^-]\ [HCO_3^-]}} \tag{12}$$

A fundamental limitation of the usefulness of such an equation has not been brought out however in their discussion. Equation (12) is derived by combining the two following equations:

$$[Ca^{++}] = 133 \frac{[H^+]}{[HCO_3^-]} \tag{13}$$

which is the maximum $[Ca^{++}]$ possible in an aqueous solution saturated with $CaCO_3$; and

$$[Ca^{++}] = \frac{(67 \times 10^{-8})}{[HPO_4^-]} \tag{14}$$

which is the maximum $[Ca^{++}]$ possible in an aqueous solution saturated with $CaHPO_4$. In discussing the use of a combined equation such as

(12) it has commonly been overlooked that such an equation is valid only when the system is saturated simultaneously with both $CaCO_3$ and $CaHPO_4$ and furthermore that under these conditions the following conditions are obligatory:

1. A fixed ratio must exist for $\frac{[HCO_3^-]}{[HPO_4^=]}$

2. The $[Ca^{++}]$ calculated from equation (13) will equal the $[Ca^{++}]$ calculated from equation (14) and also of course that calculated from equation (12).

Whenever the $[Ca^{++}]$ calculated from equation (13) differs from that calculated from equation (14) then the system is saturated only with

TABLE 3

	EXPERIMENT	$[Ca^{++}]$ EQUATION (13) (CARBONATE)	$[Ca^{++}]$ EQUATION (14) (PHOSPHATE)	TETANY
a	Before	0.41	(1.24)	
	After	(0.37)	0.09	Marked
b.	Before	0.37	(0.58)	
	After	(1.03)	0.04	None
c.	Before	0.30	(0 71)	
	After	(0.99)	0.06	None

the salt corresponding to the equation giving the lower calculated $[Ca^{++}]$, and that value represents the maximum possible $[Ca^{++}]$ in the system. Under these conditions, equation (12) is no longer valid for it is based on the assumption that the system is saturated with both carbonate and phosphate.[1] The proper use for the equations in so far as the results obtained on pure aqueous solutions of the salts can be applied to serum, would be to use equations (13) and (14) separately and take as the significant figure the lower calculated value as representing the maximum possible calcium ion concentration for the serum. This method we applied to the experimental data given in table 2 with the results shown in table 3. The values in parentheses can have, as pointed out, no real significance. With the assumptions that we are making here, the maximal calcium ion concentration after

[1] Also discussed by Robison and Soames.

injection is in each instance limited by the concentration of $HPO_4^{=}$ and there is no relation between this maximal [Ca^{++}] and the presence or absence of tetany.

It would seem therefore that the influence of pH upon the development of tetany cannot be accounted for merely by its influence upon the maximal possible calcium ion concentration. It seems probable that the pH has an additional physiological effect in influencing the susceptibility of the organism to the calcium ion concentration or to the balance of the inorganic ions.

HEMORRHAGE

Wilson (1923) has pointed out that the immediate effect of a large experimental hemorrhage is fall in blood bicarbonate, soon followed, however, by a rise above normal associated with an increase of pH above normal.

GASTRIC DISORDERS

In disease of the gastro-intestinal tract the only striking alteration observed in acid-base equilibrium is the alkalosis associated with persistent vomiting and consequent loss of acid from the body in the form of HCl. This alkalosis may give rise to tetany. MacCallum and others (1920) in experimental pyloric obstruction obtained relief of the tetany with injections of NaCl.

MacAdam and Gordon (1922) have reported the finding of an alkalosis in cases of periodic vomiting associated with definite ketonuria. This constitutes another instance of the danger of assuming a condition of acidosis to be present because acetone and diacetic acid are present in the urine. Following prolonged alkaline treatment for gastric or duodenal ulcer, Hardt and Rivers (1923) have reported toxic manifestations associated with rise in the plasma [CO_2] and with evidences of impairment of renal function.

Myers and Booher (1924) report several cases of high alkali both compensated and with high pH following Sippy treatment for ulcer. They also write "we are inclined to think that alkalosis is a condition overlooked and sometimes confused with acidosis by the clinician. We believe great care should be exercised in administration of alkali."

NEOPLASMS

In cases of carcinoma the serum ash was found by Moore and Wilson (1906) to require slightly more acid for its neutralization than normal serum and this observation was confirmed by Watson (1909). Menten (1917) reported an increase in the pH of serum of carcinoma cases, but later studies by Chambers and Kleinschmidt (1923) in which the pH was calculated from the CO_2 absorption curves and the CO_2 content of the blood as drawn with correction for oxygen unsaturation shows the averages given in table 4.

Myers and Booher (1924) in a series of 11 cases of uncomplicated carcinoma found no change in the acid base equilibrium, nor did Corran and Lewis (1924).

It seems probable therefore that there is no significant change in the serum pH in carcinoma.

TABLE 4

	AVERAGE pH
12 normals	7.29
8 miscellaneous diseases	7.33
23 carcinoma cases	7.34

RADIATION

The effect of x-ray radiation has been studied in rabbits by Hussey (1922). He observed an increase in both plasma CO_2 capacity and pH by Cullen's method, evident one hour after radiation and still present after 48 hours. The increase in pH in three hours was from 0.11 to 0.18 and in the plasma CO_2 capacity from 16 to 18 vols. per cent.

Kroetz (1924) has found in man after both x-ray and ultraviolet radiation a fall during the first hour of 3 to 10 vols. per cent in the serum bicarbonate and of 0.02 to 0.09 in pH, followed by a rise of both [$BHCO_3$] and pH the following day to about as much above the initial value. These high values may persist for a few days. These changes are consistent in all of his observations, but are obviously small.

Balderrey and Barkus (1924) have observed the effect of exposure to sunlight upon the pH of patients as measured by Cullen's (1922) method. They found no effect on cloudy days but on bright days an

increase in the average pH of 0.17. When pigmentation was marked they observed less change in pH.

Reference to figure 2 will show that the blood after administration of alkali and at a certain stage after radiation with ultraviolet light or x-ray is characterized by a change in acid base equilibrium opposite to that produced by administration of acid both as regards [$BHCO_3$] and pH. On the other hand, the rise in bicarbonate seen in certain diabetics treated with insulin shows a tendency to be associated with normal rather than with increased pH.

SURGICAL ANESTHESIA

In surgical anesthesia we have evidence of a combination of acidosis in the sense we are using the term and of depressed ventilation.

In discussions and studies of acidosis following general anesthesia with or without operation, the evidences of abnormality which have been taken as evidence of acidosis may be considered in three groups.

1. Changes in plasma bicarbonate and pH
2. Ketonuria
3. Certain clinical symptoms

Diminution in the plasma CO_2 during and immediately after ether anesthesia in man and animals has been observed by Austin and Jonas (1917) Caldwell and Cleveland (1917), Carter (1920), Collip (1920), Van Slyke, Cullen and Austin (1922), Leake, Leake, and Koehler (1923), and Austin, Cullen, Gram and Robinson (1924).

The fall of plasma CO_2 in man during operation under general anesthesia has been found to be from about 4 to 10 vols. per cent with return to approximately normal within twenty-four hours. Various types of anesthesia differ only in a minor degree in the amount of fall produced and even in operations under local anesthesia Caldwell and Cleveland observed some fall. Such changes in the alkali reserve are relatively small. In dogs under ether anesthesia the fall may be occasionally considerably greater, amounting in one instance to 22 vols. per cent (Van Slyke, Cullen and Austin (1922)), but as a rule in dogs also the fall is less than 10 vols. per cent.

The change in pH has been less extensively studied. Menten and Crile (1915) reported a fall in the blood pH in rabbits and Van Slyke,

Cullen and Austin (1922) demonstrated fall in pH in the dog under both light and deep ether anesthesia. Leake, Leake and Koehler (1923) confirm the finding. Cullen, Austin, Kornblum and Robinson (1923) have shown that this fall occurs chiefly during the early minutes of anesthesia and Austin, Cullen, Gram and Robinson (1924) have demonstrated that it is due chiefly to the introduction of some unidentified acid into the blood, together with Cl^- anions. The possibility that the unidentified acid is lactic acid had not been excluded. That lactic acid formation in the muscles is actually largely responsible for the fall in alkali reserve in the dog under ether anesthesia has now been demonstrated by Ronzoni, Koechig and Eaton and they have also discussed a possible relation of this with the observations of Stehle and Bourne (1924).

Stehle and Bourne (1924) have shown following ether anesthesia an increased phosphorus elimination in the urine, a 2.5 per cent decrease in muscle phosphorus and a 12.5 per cent increase in liver phosphorus. The change in blood phosphorus, however, is slight which taken into consideration with the base findings of Austin, Cullen, Gram and Robinson (1924) indicates that the explanation of the diminished alkali reserve is not a withdrawal of base from the blood consequent upon mobilization of muscle phosphoric acid. On the other hand the view advanced by Y. Henderson and Haggard (1918) that the fall in alkali reserve was a compensatory phenomenon consequent upon hyperpnea and excessive removal of CO_2 in the early stages of anesthesia has not been supported by the other studies mentioned.

Koehler (1924) has studied the acidosis of anesthesia as it occurs in human subjects. The changes observed are comparable to those found in experimental studies. Recovery of the acid base balance is fairly rapid and usually complete in from one to three hours. Koehler divides the recovery into two phases: that from excessive pCO_2 which is rapid occurring within the first hours and that from depressed alkali reserve which is slower.

Leake and Hertzman (1924) have studied the effects of ethylene-oxygen and nitrous oxide-oxygen anesthesia on the acid base equilibrium. They observed changes in the acid base equilibrium similar to but less marked than those observed with ether and chloroform. In addition, the factor of anoxemia is of great importance in these

types of anesthesia. There may be an initial increase in pH if the anoxemia is marked. Leake (1924) has also discussed the disturbances of carbohydrate metabolism following ether anesthesia.

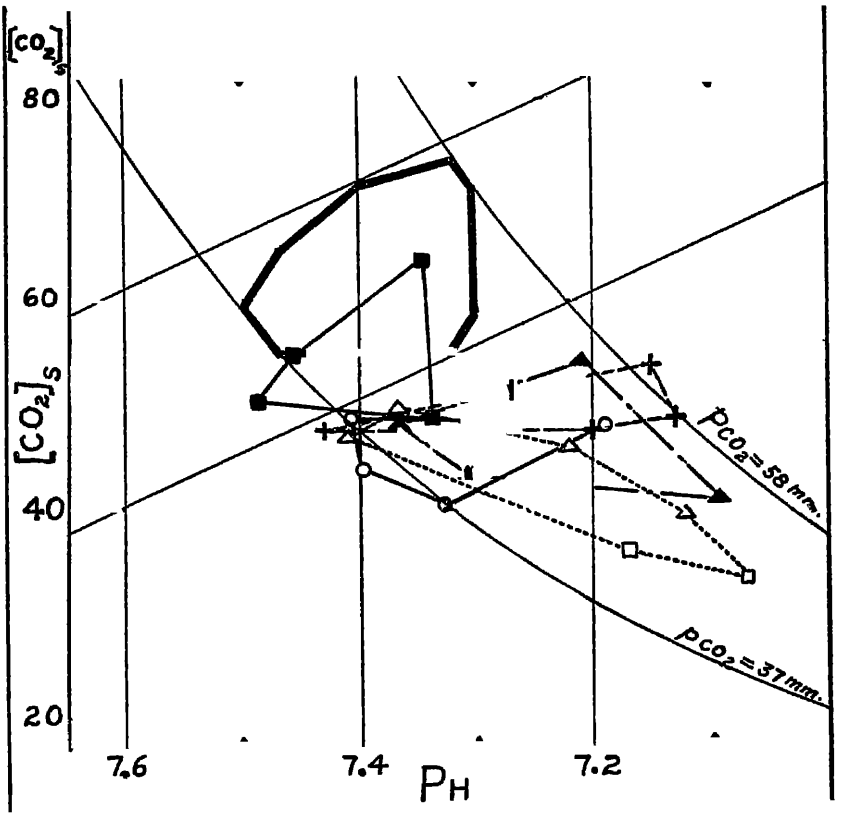

FIG. 3. VAN SLYKE TYPE OF GRAPH SHOWING NORMAL AREA OF FIGURE 1 AND EFFECTS OF ANESTHESIA

(From data of Cullen, Austin, Kornblum and Robinson (1923) in dogs and of Koehler (1924) in human cases)

■——■ Before anesthesia.
+——+ Human cases under nitrous oxide.
▲——▲ Human cases under ether.
○ ○ Human cases after nitrous oxide.
△ . △ Human cases after ether anesthesia.
□——□ Dogs after ether anesthesia.

The studies of White (1923) upon the beneficial effect of post-operative administration of CO_2 as a respiratory stimulant to accel-

erate the removal of ether through the lungs, a procedure which in itself necessarily tends for the time toward still further lowering the pH, indicate that the fall in pH is of less importance than the other effects of the ether. The procedure adopted by White led to a diminution of disagreeable post-operative symptoms.

That ketonuria as shown by qualitative tests for acetone and diacetic acid is a common finding after surgical anesthesia has long been noted and has been taken as evidence of acidosis. Reimann and Bloom (1918) and Caldwell and Cleveland (1917) demonstrated that ketonuria could often be detected immediately before operation, probably due to the preliminary fasting, but that it was much more frequent following the operation. On the other hand, they established the fact that there was no constant relation between the severity or duration of the operation or the seriousness of the post-operative course, or the degree of reducton in the alkali reserve on the one hand, and the degree of the ketonuria on the other. Indeed, it seems possible that the ketonuria is mainly an expression of the fasting incident to operation, and there is no evidence that it is of any serious clinical importance.

Of interest in this connection, however, are the observations of Thalhimer (1923), of Fisher and Snell (1924) and of Ginsberg (1924) upon the effect of insulin and carbohydrate given after operation, with a reported disappearance of ketonuria and lessening of disagreeable post-operative symptoms. The experimental studies of Stewart and Rogoff (1917) and of Ross and Davis (1920) are of especial interest in this connection.

A third group of phenomena described following operation and sometimes attributed to acidosis, probably chiefly because the common ketonuria has been examined for and noted in these cases, is a clinical picture which is essentially that of post-operative shock. The evidence that these cases actually suffer from acidosis is however lacking. These cases are not numerous in the surgical clinics and the opportunities for accurate studies of the blood are still rarer. In two such cases studied by the authors and believed by the surgeons to be post-operative acidosis, both showed plasma [CO_2] value above normal.

It seems probable therefore that grouped under the name of acidosis three more or less distinct pathological conditions have been recog-

nized as occurring at times after general anesthesia with or without operation. Of these, the one of chief clinical importance is that characterized above as a form of post-operative shock, but this condition is probably often not accompanied by acidosis and is probably not due to acidosis.

The ketonuria so often observed after operation probably arises in many instances from the fasting preceding and following operation. In itself it is of doubtful clinical importance. It may be associated with alkalosis rather than with acidosis. The value of treatment with carbohydrate with or without insulin must be based upon the effect of such treatment on the post-operative symptoms.

The fall of plasma bicarbonate and of pH, which alone should be called acidosis, appears to be a constant accompaniment of experimental and surgical ether, chloroform and nitrous oxide anesthesia, but there is no satisfactory evidence that it is of clinical importance in human surgery. When the disturbances of the acid base equilibrium that occur normally with vigorous exercise, as shown by the studies of Barr and his associates (1923) are called to mind, it may well be questioned whether the exertion incident to passing under the anesthetic may not be an important factor in this acidosis exaggerated possibly by interference under the anesthetic with normal tissue oxidation. It is not at all clear that the acidosis is a menace to the individual or that alkali therapy is indicated. With removal of the anesthetic spontaneous restoration of the acid base equilibrium is rapid as is the case after vigorous exercise. Failure of the acid-base equilibrium to recover after operation occurs probably only incident to some serious disturbance of the metabolism, and is not so far as we know a consequence of the acidosis per se.

LOBAR PNEUMONIA

In certain infectious diseases various workers have reported disturbances of the acid base equilibrium. The most thoroughly studied infection is lobar pneumonia.

Palmer (1917) demonstrated the excretion in the urine in lobar pneumonia of large amounts of organic acid which at a pH of 5.0 was present as free acid. At this pH, 87 per cent of uric acid is free, one-third of β-hydroxybutyric and of acetic but only about 5 to 7 per cent

of hippuric, diacetic and lactic acids. This fact excludes the last three from consideration as the acid excreted in pneumonia. Palmer excluded by analysis β-hydroxybutyric acid, uric acid and ethereal sulphates. The nature of the acid was not further identified. There may be a relation between these observations and those of Barach, Means and Woodwell (1922) who observed a tendency to lowering of the alkali reserve in pneumonia. They found in 10 cases an average CO_2 capacity in arterial blood at 40 mm. pCO_2 at 37° of 43.2 vols. per cent as compared with the average for normal individuals found by Peters, Barr and Rule (1921) of 49.3 vols. per cent; the lowest observed in pneumonia was 35.0 vols. per cent. Barach, Means and Woodwell calculated the arterial pH from the CO_2 absorption curve and obtained an average in their 10 cases of pneumonia of 7.31, with four cases below 7.30 which they take as the lower limit of normal resting pH. The lowest pH they observed was 7.20. They found no relation, however, between the pH or the reduction in the alkali reserve and the prognosis or the degree of anoxemia. They observed a spontaneous rise in the alkali reserve and return of pH to normal at, or shortly after, crisis in three patients studied before and after crisis. In another patient they observed the same restoration without crisis after vigorous oxygen therapy. Binger, Hastings and Neill (1923) have reported edema occurring during convalescence from pneumonia in association with bicarbonate administration. Hastings, Neill, Morgan and Binger (1924) have more recently studied a series of 30 pneumonia patients, making direct determination of the pH and $[CO_2]$. They observed a lower arterial CO_2 tension during the febrile period than after return to normal temperature in seven cases but lowered pCO_2 and increased oxygen unsaturation did not occur together with sufficient regularity to indicate a causal relationship. They noted no tendency towards an acidosis of either metabolic or respiratory origin. The alkali reserve was within or near normal limits in every case and the pH was in each instance within normal limits, 7.30 to 7.50, and in most instances in the more alkaline half of this range. Their results contraindicated alkali therapy in all the cases studied. In 8 of the 10 cases in which arterial oxygen saturation was determined an abnormally low saturation was observed at some stage of the disease. Taken with the non-occurrence of increased CO_2 tension

these results they concluded support the view that when the mechanism for gas exchange in the lungs is affected, absorption of oxygen fails before elimination of carbon dioxide is significantly impaired.

DISTURBANCES SIMILAR TO THOSE PRODUCED BY HYPOPNEA OR HYPERPNEA

Disturbances in the excretion of CO_2 either by a hyperpnea leading to a lowering of the alveolar pCO_2 or by impaired pulmonary ventilation with rise of pCO_2 produce changes in the blood that have been extensively studied. The subject has been thoroughly and extensively reviewed by Van Slyke (1921a, 1921b) with regard to the mechanism by which the pH of the blood is related to its pCO_2, and a discussion here of the development and present knowledge of this phase of the subject would therefore be superfluous. The relation of changes in pH of the blood to heart, bloodvessels and the centers of the central nervous system has been touched upon already.

In the study of disease so long as we were limited to the measurement of the plasma or blood $[CO_2]$ and to the direct measurement of the alveolar pCO_2 with its well recognized difficulties in untrained or very ill patients the recognition of disturbances in acid base equilibrium consisting chiefly of hypoventilation or of hyperventilation was difficult. With the increasing data on both plasma or blood $[CO_2]$ and direct measurement of pH of the serum there appears to be emerging evidence of disturbances of this type in certain diseases.

INFECTIONS

Hachen and Isaacs (1920) and Koehler (1923) have found in influenza, influenzal bronchopneumonia and grippe with temperatures above 103° and the latter in one case of peritonitis a reduction in the CO_2 capacity of the plasma and CO_2 content of the blood which may be rapid or gradual in its development. Koehler found the pH of the venous blood, however, increased rather than diminished and believes the fall in blood bicarbonate may be at least in part compensatory to a febrile hyperpnea, analogous to the shift in acid base equilibrium which Bazett and Haldane (1921) observed on immersion in a hot bath and which Koehler (1923) confirmed. The latter's

observations are plotted in figure 4. Yamakita (1921) however, observed a fall in alkali reserve in experimental infections even when the temperature was not very high but only with very great increase in temperature in hyperthermia due to heat puncture. Hirsch and Williams (1922) found in rabbits infected by intravenous injection of pathogenic bacteria a marked lowering of both CO_2 capacity of the plasma and of pH and Leake, Vickers and Brown (1924) observed similar changes in experimental B. bronchisepticus pneumonia in dogs. The association of the fall in [$BHCO_3$] in experimental pneumonia and other experimental infections with a lowered pH suggests that febrile hyperpnea is not always the important factor in the shift of acid base condition but that there may be depletion of the alkali reserve in certain infections. The possibility of a renal factor is to be considered in these infections.

Reports of ketonuria in the course of infections cannot be taken as evidence of acidosis. Ketonuria indicates a disturbance of the normal fat metabolism. In diabetes mellitus this is constantly associated with acidosis. Ketonuria, however, can be produced by administration of alkali as already pointed out and in the discussion of the effects of ether anesthesia we have pointed out that ketonuria is not proportional to the acidosis present.

PREGNANCY

The association of toxemias of pregnancy with increased NH_3/N in the urine has long been noted. Losee and Van Slyke (1917) showed however that in pregnant patients with pernicious vomiting and strikingly high ammonia figures the plasma bicarbonate may indicate no greater degree of acidosis than may be observed in non-toxic pregnancy. Sellards (1912) reported a similar case with only a normal tolerance for sodium bicarbonate. Losee and Van Slyke, (1917) Williamson (1923), Cook and Osman (1923), and Marrack and Boone (1923) have shown some diminution of the bicarbonate of the blood in normal pregnancy. The latter authors in seventeen cases in the last sixteen weeks of pregnancy found bicarbonate of 47 to 65 vols. per cent as compared with 58 to 65 vols. per cent for normals. Cook and Osman found the serum bicarbonate in pregnant women to be on the average 85 per cent of that for normal non-pregnant women.

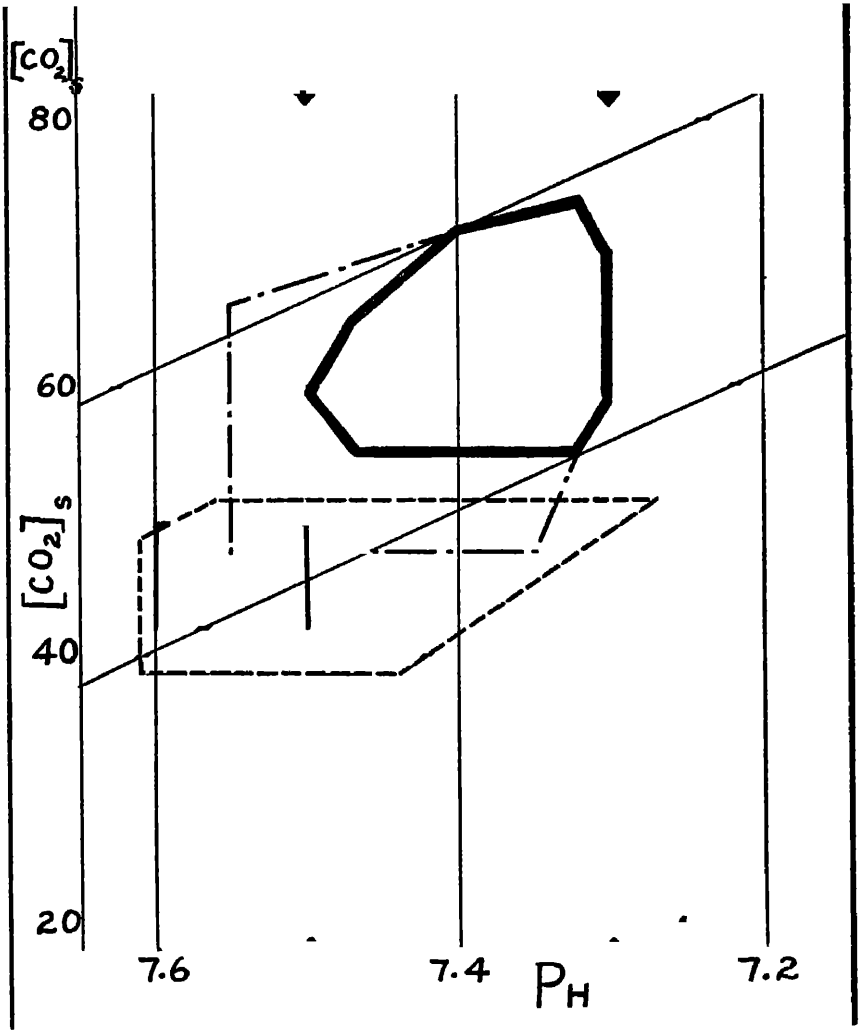

FIG. 4. VAN SLYKE TYPE OF GRAPH

Normal area of figure 1.

Area of febrile cases (Koehler, 1923).

Area of individuals in hot baths (Koehler, 1923).

Area of individuals in last sixteen weeks of pregnancy (Marrack and Boone, 1923).

The abnormal areas lie almost wholly within the normal CO_2 absorption curves but at lower CO_2 tension. (Van Slyke's "uncompensated CO_2 deficit.")

Hasselbalch and Gammeltoft (1915) noted a greater lowering in alveolar pCO_2 in the later months of pregnancy than in the bicarbonate, and Marrack and Boone observed the same. Consistent with this is the observation by Marrack and Boone of a pH of 7.35 to 7.55 by Cullen's method (1922) in their pregnant cases, as compared with 7.30 to 7.45 for normal cases (see fig. 4). This would indicate a tendency to increased pulmonary ventilation in late pregnancy, and the slight lowering of the $[BHCO_3]$ may well be considered, therefore a secondary effect of hyperventilation rather than as due to true acidosis.

CHAPTER IV

Determination of pH

The methods which have been used in studying pH of blood may be divided into three general groups. The first group includes the electrometric methods which are based upon measurement of an electrometric potential which is proportional to the H^+ concentration. The "gas chain" or "hydrogen electrode" and the "quinhydron" methods belong to this group.

The second group includes the indirect method of calculating pH either from measurement of buffer ratio or after the method of Barcroft (1914) from change in the constant of Hill's equation for the equilibrium between oxygenated and reduced hemoglobin and the oxygen tension.

The third group comprises the colorimetric or indicator methods which utilize indicators whose solutions give color effects dependent upon the pH.

The principle of these methods are reviewed fully in Clark's (1920) book.

The methods which have been of most value for investigation of blood pH are described briefly.

ELECTROMETRIC. HYDROGEN ELECTRODE

The method which has served as the reference method for all pH measurements is the hydrogen electrode or "gas chain" method which is based upon the fact that in a suitable cell the difference of potential between a metal electrode and a solution of its ions is proportional to the ion concentration (or in the newer terminology to the ion activity). Hydrogen gas, when absorbed by a platinum black electrode acts as a hydrogen electrode. This method was used by Hasselbalch and Lundsgaard (1912) in establishing the first exact value of the pH of blood. Parsons (1917) showed that the electrometric determination of pH of reduced whole blood really measures the pH of the serum of

reduced blood. Because of technical difficulties due to the presence of oxygen from the hemoglobin it is more accurate and convenient to use serum or plasma than blood. With the technic now available an accuracy of 0.01 pH may be obtained (Donegan and Parsons (1919); Warburg (1922); Cullen (1922)). Although it is the method of ultimate reference, electrometric pH determination requires so much material, time, and experience that ordinarily it is to be used only in physiological studies. It is of interest, however, that Cullen and Biilman find that with proper precautions, the quinhydrone electrode can be used with very small quantities of serum, although it can not be used with whole blood.

REFERENCE STANDARD FOR pH DETERMINATION

All hydrogen ion concentrations are referred to a hypothetical "normal hydrogen electrode." It is necessary to base the actual determination upon some reproducible standard of reference. The recent development in the knowledge of electrolytic dissociation and the use of the activity coefficient for hydrogen ion activity has led to some confusion. The reference standard is relatively unimportant in any investigation where only changes of pH are being studied, but becomes of importance when comparing results from different laboratories on such questions as normal pH of blood.

As pointed out later the relation between the pH at different temperatures is also confused in this question of reference standard.

Clark (1922) and Soerensen and Linderstroem-Lang (1924) have agreed that all biological hydrogen ion concentrations shall be reported by them in terms of the older pH values based on conductivity instead of the newer pH activity values.

They also agree that the standard of reference shall be the N/10 calomel cell to which they have assigned definite values at various temperatures. The present authors prefer to standardize their pH determinations against a reproducible standard acid solution either 0.1N HCl or 0.01N HCl in 0.09N KCl, and to assume no change in pH of this standard solution with change in temperature (see Cullen, Keeler and Robinson (1925)).

With the latter standardization, Soerensen's phosphate standards have his values at 18° and are 0.03 pH less at 38°, i.e., a phosphate

solution which is 7.40 at 20° is 7.37 at 38°. Our pK′ values for serum based on this system and using Bohr's α values are 6.18 at 20° and 6.10 at 38°, giving a temperature difference in agreement with other workers using the N/10 calomel cell standard.

CALCULATION OF pH FROM [$BHCO_3$] CONTENT AND CO_2 TENSION

This method using equation 17 requires determination of total CO_2 content, and of CO_2 tension. The total [CO_2] is determined most easily and accurately by Van Slyke's CO_2 apparatus (Van Slyke and Neill, 1924) and the pCO_2 may be determined either by alveolar CO_2 determination (when the subject can coöperate) or from the CO_2 absorption curve. For the latter purpose the total CO_2 content is determined on one portion of the blood or serum as drawn, and other portions are equilibrated with known CO_2 tensions at two or more points. Analysis of total CO_2 of these equilibrated samples gives data for the CO_2 absorption curve. Peters, Bulger and Eisenman (1924) have recently proposed the construction of the curve from one point (see our equations (20) and (21)). The interesection of this curve with the CO_2 content of the original blood or serum gives the CO_2 tension (pCO_2) as drawn. If venous blood is used it is necessary to correct the total [CO_2] for the change in [$BHCO_3$] with change in oxygenation of hemoglobin (see equation (22)).

This method, which has been extensively used requires appropriate pK′ values for blood and for plasma or serum. The pK′ values for serum have been recently redetermined for serum, for a variety of clinical conditions (Cullen, Keeler and Robinson, 1925). The value of 6.10 at 38° and 6.18 at 20° are reliable for serum and plasma (see also Warburg (1922) for review of previous work).

The difference between pK'_{serum} and pK'_{blood} used to calculate the pH of serum since the pCO_2 is the same, must be dependent entirely upon the different solubility of CO_2 in whole blood and serum and upon the difference in [$BHCO_3$]. This relation as pointed out above is influenced by cell volume (hemoglobin content) and pH of the serum. This difference, $pK' - pK'_s$, has been recently studied by Warburg (1922), by Peters, Bulger and Eisenman (1924), and by Van Slyke, McLean and Wu (1923). The studies including that of Peters, Bulger and Eisenman on a large series of human bloods (see equation

(21)) are in close agreement with each other. Hastings (quoted by Van Slyke, Wu and McLean, 1923, p. 800) has represented the relation on a D'Ocagne-Henderson line chart. The values for pK'_s we give in table 5, and for pK'_3–pK'_s in figure 5.

The technic which we employ for equilibration to known CO_2 tension and for calculation of data is summarized in a previous paper (Austin *et al.*, 1922).

COLORIMETRIC DETERMINATION OF pH

Because of its simplicity and economy of material and time the direct determination of pH on blood plasma is the method of choice for clinical investigation. The principle of the colorimetric method involves (1) handling of blood and plasma without loss of CO_2, (2) either elimination of protein error by dialysis or correction for the protein error of the indicator. The colorimetric and electrometric methods applied to protein free salt solutions are in perfect agreement when sufficient precautions are used to prevent loss of CO_2 (Cullen and Hastings, 1922).

The blood may be dialyzed against neutral saline solution and the pH of this dialysate determined by use of an indicator. Levy, Rowntree and Marriott (1915) used this method and introduced the use of phenolsulphonephthalein (phenol red) as indicator. These authors did not prevent loss of CO_2 so that their values did not represent actual pH. Dale and Evans (1920) modified this method by preventing loss of CO_2. Dale and Evans used neutral red and titrated a phosphate control to the same color. Lindhard introduced a micro-modification of Dale's and Evan's method.

Cullen (1922) determined directly the pH of the plasma diluted 1:20 with saline and determined by comparison with electrometric determination the total empirical correction for dilution, salt, protein and temperature. This method has proved convenient for both clinical and physiological studies.

Blood is drawn *without stasis or loss of CO_2* into a tube under paraffin oil containing oxalate to make 0.3 per cent. It is centrifuged in a stoppered tube completely filled with blood. Especial care is taken that neither the blood or plasma is ever exposed to air. One portion of plasma of from 0.2 to 1 cc. is transferred to 20 volumes of 0.9 per

cent NaCl solution containing phenol red, which is already covered with oil. Another portion is added to 0.9 per cent NaCl solution without indicator. The indicator NaCl solution is prepared by adding 1.05 cc. of 0.04 per cent phenol red to 100 cc. NaCl solution and adjusting with N/50 NaOH to pH about 7.5. Phosphate standards at 0.05 pH intervals are used containing 0.01 cc. of 0.04 per cent phenol red per cubic centimeter. The second sample of plasma in saline is used to superimpose the color of the serum upon that of the standard with indicator. The color of the plasma + indicator tube is compared with the combined colors of phosphate + indicator and diluted plasma in a Walpole comparator. For human plasma

$$pH_{38°} = pH_{color} \text{ at } t° + 0.01\ (t° - 20°) - 0.23 \qquad (15)$$

Where "pH_{38}" is the pH of the undiluted plasma at 38°; "t°" is the temperature (15° to 25°) of the phosphate standards and diluted serum when read; "pH_{color}" is the pH at 20° of the phosphate standard which matches the plasma + indicator tube.

Hawkins (1923) has found that the whole blood can be added directly to the saline before centrifuging, thus 0.25 cc. + 5 cc. saline. The empirical correction "C" given in equation (15) as −0.23 varies with different species. The value for normal human plasma was found by Cullen (1922) to be −0.23 ± 0.04. The extent to which this correction varies under pathological conditions has not however been adequately determined. Not only is the average value for other species different but the individual variation in some species, such as the dog, is apparently greater than in the human.[1] Hastings, Neill, Morgan and Binger (1924) found the average value for twelve pneumonia patients to be −0.27 ± 0.05. Recently Hastings and Sendroy (1924) report that reading both standard and diluted plasma at 38° eliminates this correction. We have found this to be true in some sera but not in all. For the present we must look upon the magnitude of this correction whether the reading be made at 20° or at 38° as open to further investigation. *Calculation of CO_2 tension* may be made directly when total CO_2 and pH determinations have been made.

[1] Attention was first called by M. A. Bennett (1925) to the importance of the variation in this correction. These variations were subsequently further studied by Austin, Stadie and Robinson (1925).

SUMMARY OF EQUATIONS AND CONSTANTS FOR EXPRESSING RELATIONS BETWEEN CERTAIN FACTORS IN THE ACID BASE EQUILIBRIUM

It seems desirable for convenience to gather together at this point certain relations between factors concerned in the acid base equilibrium in the blood and used in the calculation of one from another.

The Henderson-Hasselbalch equation for the relation between pH of serum or plasma and CO_2 tension and CO_2 content of the serum or plasma is:

$$pH_s = pK'_s + \log \frac{[BHCO_3]_s}{[H_2CO_3]_s} \tag{16}$$

This may also be written

$$pH_s = pK'_s + \log \frac{[CO_2]_s - 0.1316\,\alpha_s pCO_2}{0.1316\,\alpha_s pCO_2} \tag{17}$$

where for convenience, we express CO_2 tension in millimeters, instead of atmospheres so that pCO_2 = mm. CO_2 tension and $0.1316\ \alpha = \frac{1}{760}\alpha$. $[CO_2]_s$ = $[CO_2]$ (at 0°, 760 mm.) of serum or plasma in volumes per cent.

TABLE 5

t°	0.1316 α water	0.1316 α_s (BOHR)	0.1316 α_s (V. S. W. M.)
15	0.1340	0.1307	0.1225
20	0.1156	0.1128	0.1057
25	0.0999	0.0975	0.0913
30	0.0875	0.0855	0.0800
38	0.0730	0.0712	0.0668
45	0.0630	0.0614	0.0576
pK'_s at 38°		6.10	6.07

The values for α that have been employed in the past for serum or plasma are based on the observation of Bohr that $\alpha_s = 0.975\ \alpha_{water}$. More recently Van Slyke, Wu and McLean (1923) have found that the ratio of α_s to α_{water} is proportional to the concentration of water in the serum and they have found the average water content of serum to be 91.4 per cent. In table 5, we give both these values for α_s and the corresponding values for pK'_s. In figure 7 we have plotted only Bohr's values for $0.1316\ \alpha_s$ since the pK'_s values in that figure

are calculated accordingly. The value for pK'_s for human serum or plasma at 38° we take as 6.10 with Bohr's α_s (Cullen, Keeler and

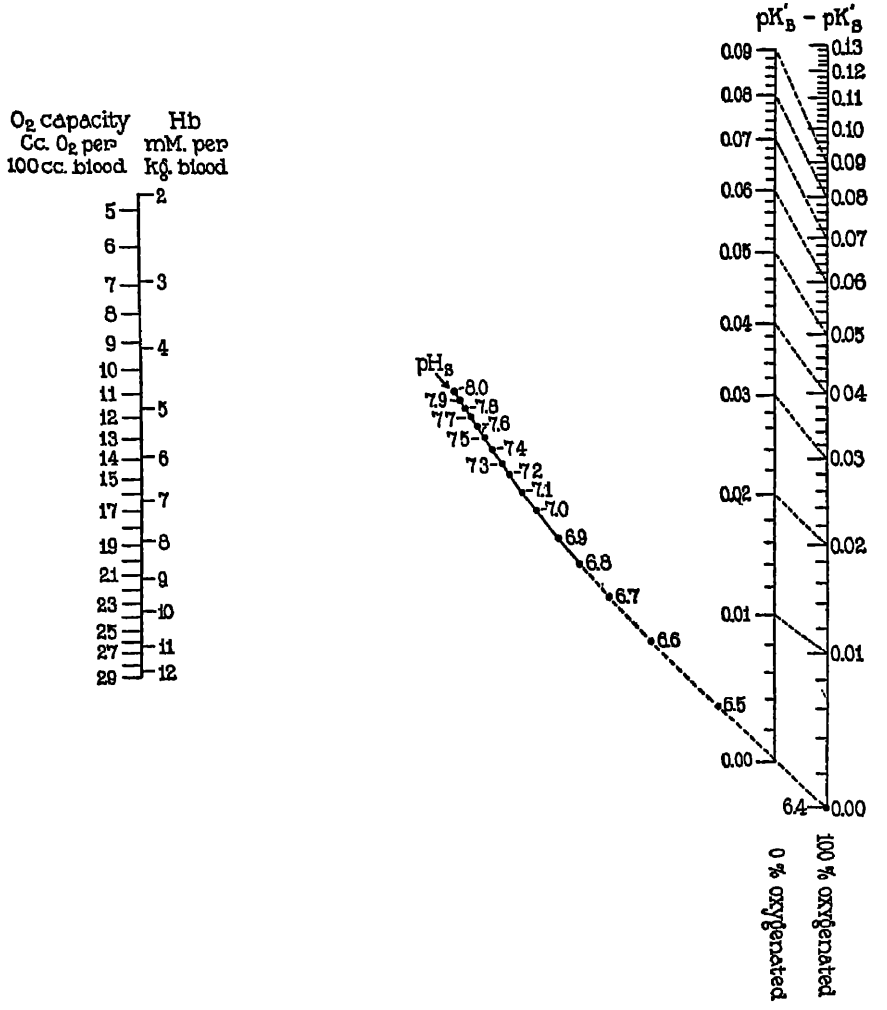

FIG. 5

By the courtesy of Drs. D. D. Van Slyke and A. B. Hastings were are permitted to include this graph prepared by them as a modification of figure 6b (Van Slyke, Wu, McLean, 1923). It permits the reading of $pK'_B - pK'_s$ for any oxygen capacity and pH_s for both fully oxygenated and fully reduced blood with values at intermediate oxygenation by interpolation. A straight line drawn through the known O_2 capacity (or Hb) and pH of serum values will intersect the pK–pK lines at the appropriate correction values.

Robinson (1925)) or with the lower value for α_s of Van Slyke, Wu and McLean the corresponding pK'_s is lower by 0.028 or in round numbers 6.07.

The Henderson-Hasselbalch equation for the relation between pH of serum and the CO_2 tension and CO_2 content of whole blood is:

$$pH_s = pK'_B + \log \frac{[CO_2]_B - 0\ 1316\ \alpha_B pCO_2}{0.1316 \alpha_B pCO_2} \qquad (18)$$

Constant values for pK′ and α_B have commonly been employed in the past differing from pK'_s and α_s by a constant increment. Since, however, these constants for blood differ from those for serum by an increment related to the proportion of cells in the blood, they can be stated to advantage as a function of the O_2 capacity of the blood.

For this purpose we follow Van Slyke, Wu and McLean (1923) using their equation (30). This gives:

$$\alpha_B = (1 - 0.0067\ h)\ \alpha_S \qquad (19)$$

Where h = oxygen capacity in volumes per cent. Using equation (19) to express the relation between α_s and α we take as values for pK′ –pK'_s the graph, figure 6b, from Van Slyke, Wu and McLean (1923). These values they point out agree with the data of Peters, Bulger and Eisenman (1923).

By the courtesy of Drs. D. D. Van Slyke and A. B. Hastings we include a revised form of this graph as figure 5.

The use of CO_2 content and CO_2 tension of whole blood and equation (18) to calculate pH_s is open to much more uncertainty in the values of the constants of the equation than is the use of CO_2 content of serum and equation (17). The latter course is to be preferred wherever possible; this is especially true when blood from different species is under study.

In the study of the carbon dioxide absorption curve of blood we formerly required a knowledge of the CO_2 content of a given sample of blood at three known CO_2 tensions or at three pH values in order to plot the CO_2 absorption curve. Subsequently, however, it was shown by L. J. Henderson (1921) and Warburg (1922) that with changing pCO_2, $[BHCO_3]$ of blood plasma plotted against its pH is

approximately linear. Barcroft, Bock, Hill, Parsons, Parsons and Shoji (1922) showed that under the same conditions $[CO_2]$ plotted against $[H^+]$ is approximately linear and Peters, Eisenman and Bulger (1923) showed that under the same conditions log $[CO_2]$ plotted against log pCO_2 is linear. Each of these methods make possible the determination of the CO_2 absorption curve from a knowledge of CO_2 content and either pCO_2 or pH at two CO_2 tensions. Finally for human blood Peters, Bulger and Eisenman (1924) have shown that the slope of log $[CO_2]$ against log pCO_2 can be approximated if the oxygen capacity be known, as follows:

$$\Delta [CO_2]_{60-30} = 0.334\,h + 6.3 \qquad (20)$$

where "$\Delta[CO_2]_{60-30}$" is the increase in CO_2 content in volumes per cent between $pCO_2 = 30$ mm. and $pCO_2 = 60$ mm., and "h" is the oxygen capacity expressed in volumes per cent. This permits the approximation of the CO_2 absorption curve for human blood from the knowledge of the CO_2 content at one pCO_2.

The level of true serum absorption curve above the blood curve at 38° has been found by them to be at $pCO_2 = 40$ mm.

$$[CO_2]_S = [CO_2]_B + (0.0159\,[CO_2]_B - 0.281)\,h \qquad (21)$$

Using equations (17), (18), (19) and figure 5 one can calculate $[CO_2]_S - [CO_2]$ for a given blood at any CO_2 tension and the result may be compared at $pCO_2 = 40$ with the value obtained by using equation (21). The calculated values for $[CO_2]_S - [CO_2]_B$ differ considerably, which is probably not surprising when the widely different kind of data on which the various constants are based is considered. The discrepancy emphasizes the need for further experimental study of the relation between blood and serum already pointed out by the authors of these equations in order to define the applicability and limitations of the relations expressed in equations (18), (19), (21) and figure 5. The slope of the true serum curve may be approximated from that of the blood curve by assuming that $[CO_2]_S - [CO_2]_B$ is the same at 30 and 60 mm. pCO_2 as at 40, or by assuming that $\log \frac{[BHCO_3]_S}{[H_2CO_3]_S} - \log \frac{[BHCO_3]_B}{[H_2CO_3]_B}$ is 0.01 less at $pCO_2 = 60$ and 0.01 more at $pCO_2 = 30$ than at $pCO_2 = 40$. The result obtained by either method is substantially the same and is approximately con-

sistent with the observed relations. The slope of the true serum curve can also be approximated by equation (20) for whole blood, given above.

When the $[CO_2]_B$ and $[O_2]_B$ of blood as drawn is known and in addition the oxygen capacity and CO_2 absorption curve of the blood, fully oxygenated has been determined at body temperature in vitro and it is desired to determine the CO_2 absorption curve of the blood at the state of oxygen saturation as drawn, we may use the formula of Doisy, Briggs, Eaton and Chambers: at any pH (pH = X):

$$\frac{[CO_2]_B - [CO_2]_{sat.}}{[O_2]_{sat} - [O_2]_B} = 0.44 \qquad (22)$$

where $[O_2]_{sat}$ = oxygen capacity.
$[O_2]_B$ = oxygen content in the state of partial unsaturation.
$[CO_2]_{sat}$ = CO_2 content of blood, at pH = X, when saturated with oxygen.
$[CO_2]_B$ = CO_2 content of blood, at pH = X, when at the oxygen saturation indicated by $[O_2]_B$.

$[CO_2]$ and $[O_2]$ must be expressed in the same units (volumes per cent, mM., etc). The CO_2 absorption curve of the oxygenated blood can then be raised at each pH to a level corresponding to that for the state of unsaturation as drawn and the $[CO_2]_B$ as drawn interpolated on this curve to obtain the pH as drawn, whence the pCO_2 can be calculated.

Logarithmic paper ruled as suggested by Peters (1923) with pH lines is very useful in plotting CO_2 absorption curves. Such a chart can be prepared for serum but not to advantage for whole blood since for its preparation a constant value for α is necessary when pCO_2 is used as abscissae and also a constant value for pK', but both α_B and pK'_B vary in different bloods according to the oxygen capacity.

TEMPERATURE EFFECT

In the biological studies thus far made, interest has centered almost exclusively upon the cause and effect of changes in $[H^+]$ under conditions of constant temperature or where at least change in temperature was not an essential feature of the conditions being investigated. This has been of importance, sometimes unrecognized, in

view of certain limitations in the measurements of $[H^+]$ and in view of the relation between $[H^+]$ and $[OH^-]$.

For electrometric measurement of $[H^+]$ at any given temperature a value for $[H^+]$ of some standard solution must as we have already pointed out be more or less arbitrarily assigned for the temperature in question; then at the same temperature the ratio to this of $[H^+]$ of any other solution having been measured, its $[H^+]$ can be calculated with reference to the standard chosen. The various standards in use in this connection we have already discussed.

The direct measurement with the gas chain of an unknown $[H^+]$ at one temperature against a standard $[H^+]$ at another temperature is without any physical significance and cannot be interpreted. For each temperature employed with the gas chain a new value for the $[H^+]$ of the standard solution or cell must therefore be assumed, or calculated in accordance with some assumption, and no one basis for this assumption has been generally accepted in biological work. The relation of pH = 7.40 at 20° to pH = 7.40 at 38° is one, therefore, that can be stated only when one has defined the standard values employed at each temperature.

The difficulty just stated is one of standardization of measurement. There is, however, a second factor to be considered in dealing with varying temperatures. In aqueous solution $[H^+] \times [OH^-] = K_w$ and at any given temperature, K_w is constant. At constant temperature any increase in acidity always signifies a proportionate decrease in alkalinity. In considering the biological significance of a change of reaction we as a rule make no attempt to distinguish the importance of change in $[H^+]$ and $[OH^-]$ respectively, and at constant temperature such a distinction is of no great significance since they are inversely proportionate.

When we deal with changing temperature the situation alters, however. With change in temperature K_w changes. With change in temperature it would be possible to have simultaneous increase in both $[H^+]$ and $[OH^-]$; change in one no longer implies a reciprocal change in the other. Under these conditions in considering reaction we have three distinct factors we may consider

$$(1)\ [H^+],\ (2)\ [OH^-],\ (3)\ \frac{[H^+]}{[OH^-]}.$$

When the ratio of $\frac{[H^+]}{[OH^-]} = 1$, the condition is by definition one of neutrality.

The values for K_w, that is for the product $[H^+] \times [OH^-]$, at different temperatures have been calculated by Lewis and Randall (1923, p. 487) on the basis of Wörmann's determinations of heats of neutralization of strong acids and alkalies at various temperatures. Their

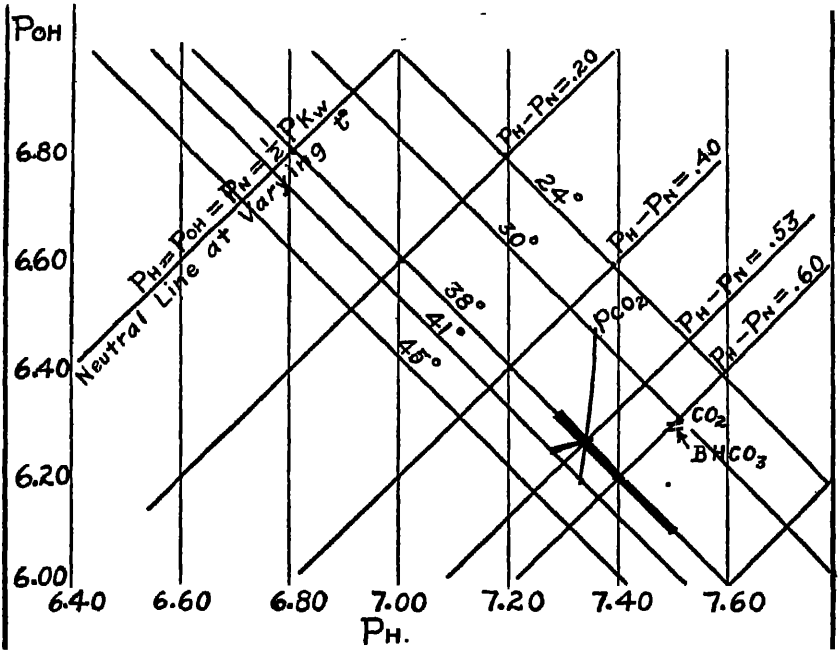

FIG. 6. pH AGAINST pOH AT VARYING TEMPERATURES

The line of neutrality where pH = pOH at varying temperature.

The shaded area is the normal pH, pOH and temperature range of serum.

The curves show the effect of changing the temperature of a particular blood and its true serum at constant pCO_2, at constant $BHCO_3$ and at constant total CO_2.

values have been used in construction of figure 6, which illustrates the relationships of $[H^+]$ and $[OH^-]$ at varying temperatures. It will be seen from figure 6 that a serum which at 38° has a pH of 7.40 and a pOH of 6.20 has the same value for pH-pOH and a pH equally removed from the neutral point as a serum at 41° with a pH of 7.36 and a pOH of 6.16.

In biological studies under conditions of varying temperature we must determine, therefore, whether the significant factor is $[H^+]$ or $[OH^-]$ or the ratio of $\frac{[H^+]}{[OH^-]}$ and our logarithmic notation will be

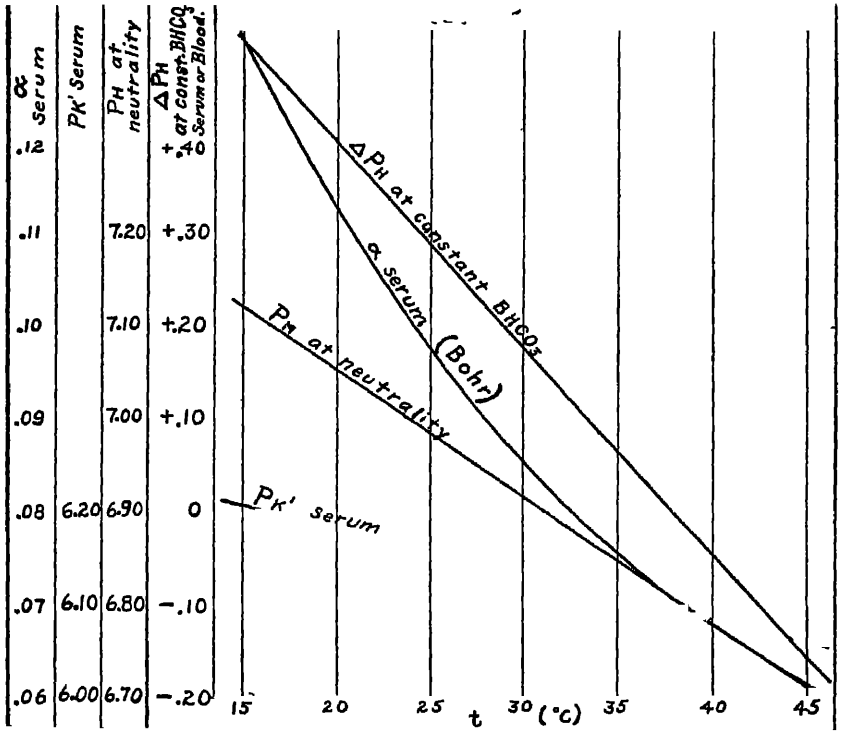

FIG. 7

(1) α-serum values in above figure are those (Bohr) given in table 5, i.e. 0.1316 and are used in the equation:

$$[H_2CO_3] \text{ (vols. per cent)} = 0.1316\ \alpha\ pCO_2$$

(2) pK' serum in equation:

$$pH = pK'_s + \log \frac{[BHCO_3]_s}{[H_2CO_3]_s} = pK'_s + \log \frac{[CO_2] - 0.1316\alpha pCO_2}{0.1316\alpha pCO_2}$$

using Bohr's α.

(3) pN = pH at neutrality = pOH at neutrality. pOH = 2pN − pH.

(4) Δ pH at constant $[BHCO_3]$, permits construction of CO_2 absorption curve at changed temperature.

accordingly pH or pOH or 1/2 [pH−pOH], the latter expression measuring the increment of pH to the acid or alkaline side of the

neutral point at the temperature in question. Clark (1920) has pointed out that the neutral point although a convenient point of reference is not as a rule characterized by any striking change in chemical behavior. We may, however, expect to find in some reactions the major importance attaching to pH and in others to pOH.

In the study of blood acid base equilibrium under conditions of varying temperature there are four variables of which the temperature coefficient must be taken into account.

1. The solubility of CO_2. The solubility of CO_2 in serum is given in table 5 and figure 7. So far as we know equation (19) is applicable at any temperature under consideration.

2. The value of pK'_s at varying temperatures has been recently determined by Cullen, Keeler and Robinson (1925) and the result of their observations is given in figure 7. The observed change with temperature is only a little more than half that calculated by L. J. Henderson (1908) and by Stadie and Martin (1924) from the data in the literature upon the heat of ionization of H_2CO_3. The explanation for this discrepancy is not clear; possibly it lies in the choice of values for standardization of pH measurements at varying temperatures. In any case, however, the values shown in figure 7 are those which are consistent with our present methods and standardization in blood equilibrium studies.

3. Change in the location of the CO_2 absorption curve occurs due to different heats of ionization of protein acids and H_2CO_3. Or stated in other words the shift of the CO_2 absorption curve is due to unequal changes in the pK'_1 and pI of equation (4a). Experimental data on the magnitude of this change is very scanty. Stadie and Martin have data which indicate that the CO_2 absorption curves of whole blood are approximately parallel at 38° and at 15° and that the change in pH at the same $[BHCO_3]$ is given approximately by the equation

$$\Delta pH_{[BHCO_3]} = 0.02\ (38° - t°) \qquad (23)$$

Stadie, Austin and Robinson (1925) have found this factor to be 0.02 ± 0.003 for both whole blood and true serum in the dog and sheep. Stadie has observed the change for separated serum in the dog and sheep to be within these limits.

The advantage of considering the change in location of the CO_2 absorption curve with change in temperature in terms of change of pH at constant $[BHCO_3]$ may be explained as follows:

There is in a given sample of blood or serum a certain amount of base combined with strong acids (chiefly Cl'). The amount of base bound by these acids is not significantly effected by change in temperature. The remaining base is bound by weak acids, chiefly protein acids and HCO_3', in accordance with equation (4a). The values for pK_1' and pI in this equation vary with temperature and unequally. If as an approximation we neglect the phosphates and assume that the base free to combine with weak acids is given by $[BHCO_3] + [BPr]$ it follows that when under conditions of changing temperature we keep $[BHCO_3]$ of a sample of blood or serum constant we will also keep [BPr] constant. Under these conditions change in pH or $[H^+]$ is related to change in pI with change in temperature as follows:

$$\log \frac{[H^+]_t}{[H^+]_{t'}} = pI_{t'} - pI_t$$

or

$$pH_t - pH_{t'} = pI_t - pI_{t'}$$

Change in pH with temperature at constant $[BHCO_3]$ is therefore a measure of the change of pI of protein with temperature. It is reasonable to expect a reasonable constancy in the change of pI of blood proteins with temperature and hence of pH at constant $[BHCO_3]$ as indicated in equation (23).

Accepting the value of 0.02 for $\Delta pH_{[BHCO_3]}$ tentatively for the present we indicate in figure 7 its magnitude at varying temperatures. It must be pointed out that if the pH at constant $[BHCO_3]$ with varying temperature is approximately the same for separated serum, true serum and whole blood, the $\Delta [BHCO_3]$ at constant pH with varying temperature will be much less for separated serum than for true serum or for whole blood due to the difference in the slope of their respective buffer curves.

The change in $[BHCO_3]$ with change in temperature at constant pCO_2 or at constant $[H_2CO_3]$ can be readily calculated from equation

16 and from the CO_2 absorption curve if the slope of the latter is known or can be approximated.

This relationship is shown in table 6.

4. At constant temperature $\Delta pH = -\Delta pOH = -\Delta(pN-pH)$ where pN = pH at neutrality = pOH at neutrality = 1/2 pK water. The variation of pN with temperature (from pK_w calculated by G. N. Lewis) is shown in figure 7. At any temperature pOH = 2 pN−pH.

TABLE 6

Effect of change in temperature on pH of true serum and separated serum various factors being kept constant

(Calculated from equations (23) and (16))

TEMPERATURE	ASSUMED CONDITION	$[CO_2]$	pCO_2	$[H_2CO_3]$	$[BHCO_3]$	pK′	pH	ΔpH	log R
True serum $\frac{d[BHCO_3]}{dpH} = -62$ (t° constant)									
		vols. per cent	*mm.*	*vols. per cent*	*vols. per cent*				
38°	Initial	54.1	43.3	3.07	51 0	6.100	7.320		1.220
41°	Constant $[BHCO_3]$	54.4	51.2	3.41	51.0	6 085	7.260	−0 060	1.175
41°	Constant pCO_2	51.0	43.3	2.88	48 1	6.085	7.308	−0.012	1.223
41°	Constant $[H_2CO_3]$	52.3	46 0	3 07	49.2	6.085	7.290	−0.030	1.205
Separated serum $\frac{d[BHCO_3]}{dpH} = -14$ (t° constant)									
38°	Initial	54.1	43 3	3 07	51.0	6.100	7.320		1.220
41°	Constant $[BHCO_3]$	54.4	51.2	3 41	51 0	6 085	7.260	−0.060	1.175
41°	Constant pCO_2	53 0	43 3	2.88	50 1	6 085	7.325	+0.005	1.240
41°	Constant $[H_2CO_3]$	53 5	46 0	3.07	50 4	6 085	7.300	−0.020	1.215

Therefore with change in temperature between 15° and 45° there is at constant pH the following change for each rise of 1°C.

$$\Delta (pN - pH) = -0.0143.$$

$$\Delta pOH = -0.0286$$

Taking these four changes into account the relations for a particular true serum at 30°, 38° and 41° are shown in figures 6 and 8. The CO_2 absorption curves in figure 8 represent the approximate calculated change in the true serum from the blood of W. C. S. from the data of

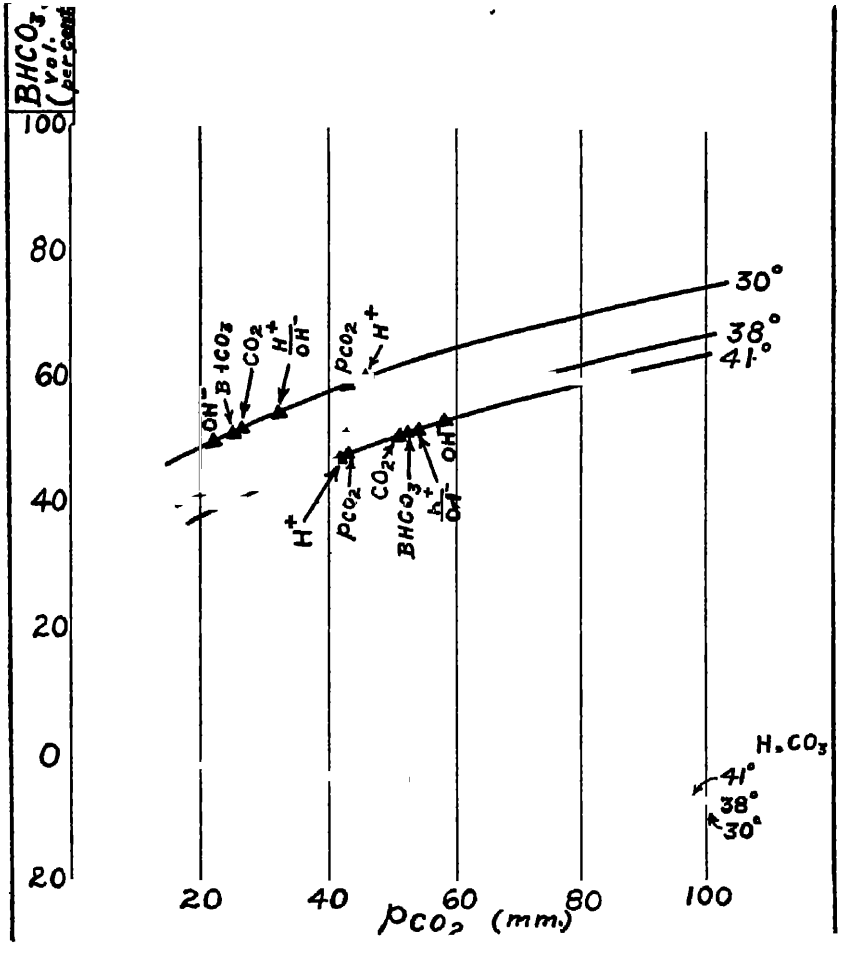

FIG. 8. THE CO_2 ABSORPTION CURVES FOR TRUE SERUM OF THE SAME BLOOD AT THREE TEMPERATURES ARE SHOWN

An initial point is indicated on the 38° curve and on the other curves are indicated the points which have the same pH, pOH, $p\frac{(H^+)}{(OH^-)}$, $[BHCO_3]$, $[CO_2]$ and pCO_2 as the initial point.

Stadie and Martin (1924). It will be seen that when the blood temperature is increased from 38° to 41° and the CO_2 tension is kept constant the following ensue:

Slight reduction of pH (slight increase of $[H^+]$)
Marked reduction of pOH (marked increase of $[OH^-]$)
Marked decrease in the ratio $\frac{[H^+]}{[OH^-]}$ (pOH − pH)
Decreased CO_2 capacity.

Under these conditions fall in pH (increase in $[H^+]$) does not mean diminished alkalinity.

These relations are also shown in figure 6. It will be seen that change in temperature at constant pCO_2 causes little change in pH but marked change in pOH. On the other hand change in temperature at constant $[CO_2]$ or $[BHCO_3]$ causes marked change in pH and little change in pOH. These data permit us to apply our methods of calculating to the case of febrile blood or to blood from chilled or heated extremities.

When blood is obtained at a temperature other than 38° and its $[CO_2]$ determined and in addition its CO_2 absorption curve at 38° is determined the following corrections must be introduced to determine pCO_2 and pH as drawn:

1. Relocation of curve for unsaturation (equation (22))
2. Relocation of curve for temperature (equation 23 or Δ pH at constant $BHCO_3$ from figure 7)
3. Use of α_s pK'_s and pN for appropriate temperature (see figure 7).

When blood is obtained at a temperature other than 38° and its $[CO_2]$ determined and its pH at 38°, the correction to original pH is given by equation 23. This equation is at present an approximation only and can therefore be used for constant $[CO_2]$ as well as for constant $[BHCO_3]$.

We are indebted to Mr. H. W. Robinson for assistance in the preparation of the graphs.

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